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## A STUDY IN THE MINERAL COMPOSITION OF THE SOILS OF SOUTH AYRSHIRE

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(With Five Text-figures)

### INTRODUCTION

THE present investigation was carried out in connection with a Soil Survey of Southern Ayrshire by the staff of the Department of Chemistry, West of Scotland Agricultural College, under Prof. D. N. McArthur.

The area covered by this survey is that embraced by the Geological Survey Map of Ayrshire, Sheet 14, and includes Monkton and Tarbolton in the north, Kirkoswald, Straiton and Dalmellington in the south, and stretches as far east as Cunnoch. It amounts to nearly 400 square miles, and includes one of the best dairy-farming districts and some of the finest agricultural land in Scotland. The higher ground is mainly devoted to sheep rearing.

### PHYSIOGRAPHY (7,9)<sup>1</sup>

The greater part of the area is hilly, the highest portions being in the south and east, where, at one point, a height of over 1800 ft. above sea-level is reached.

In the northern half there is a broad, low-lying coastal belt, which slopes gently upwards from sea-level to the high ground in the east and south-east. The two most important rivers of this northern part are the Ayr and the Lugar, both of which flow through deep and often narrow valleys.

In the southern half the land rises rapidly from the coast, and heights of from 500 to 900 ft. are common less than 3 miles inland. The steep western wall of the gap in which Maybole and New Dailly are situated forms the eastern boundary of these hills, while the gap itself separates them from the higher ground farther inland. In this high land there rise two of the important rivers of south Ayrshire, the Doon and the Girvan. Here also are the head waters of the Nith, which, however, flows east and then south to the Solway Firth. All these rivers occupy deep and wide

<sup>1</sup> See numbered references at close of paper.

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valleys, enlarged by glacial erosion, and which form conspicuous scenic features.

The climate is of the moist, humid type with the prevailing wind from the south-west. On the low-lying coastal parts the average rainfall is from 30 to 40 in. per annum, while on the higher ground farther inland the average is from 40 to 60 in.

### GEOLOGY (9)

#### (i) *Solid*

The formations and rock types entering into the structure of the district are:

Recent: Blown sand, peat, alluvium, raised beaches.

Glacial: Sands and gravels, moraines, boulder clay.

Permian: Sandstones and lavas.

Carboniferous: Sandstones, shales, limestones, lavas.

Old Red Sandstone: Sandstones and lavas.

Silurian: Sandstones, shales, greywackes.

Ordovician: Sandstones, conglomerates, greywackes, limestones, lavas.

Igneous intrusions of Tertiary, Permo-carboniferous and Old Red Sandstone age.

The Ordovician rocks, part of the broad belt of the Southern Uplands, occur mainly in the south-east corner and form the high ground there. They consist chiefly of shales, grits and greywackes. Inliers, both of Ordovician and Silurian sediments, occur nearer the coast, notably to the north of Craighead in the south-east corner of Sheet 14, west of New Dailly. Here Silurian deposits surround and overlie Ordovician rocks, the latter including lavas of Arenig age.

The predominant rock, however, in the southern half of the area is the Old Red Sandstone, both Lower and Upper, the former with associated andesitic lavas. These lavas also form high ground, for example, the Brown Carrick Hill to the south of Ayr.

In the south-west corner and forming the eastern boundary of the Craighead inlier of Ordovician and Silurian rocks already referred to, is an outlier of Lower Carboniferous sediments.

Also in this part, and of Old Red Sandstone age, are a few small acid intrusions, mainly felsitic in character.

The Old Red Sandstone rocks and the Carboniferous outlier, generally speaking, lie south and west of a line drawn from the north end of Loch Doon to the mouth of the River Doon near Ayr. North and east of

this line more than half of the rocks at the surface are of Carboniferous age. All divisions of this system, from the Calciferous Sandstone to the Barren Red Measures, are represented, especially the Coal Measures.

In addition to these, the Permo-carboniferous suite of basic intrusions is well represented, and includes dolerites, teschenites and allied rocks.

The Mauchline Permian Basin is situated in the northern part of the area, and includes a series of basaltic lavas covered by a thick development of desert sandstone.

Immediately outside the area in the south and south-east is the broad, high belt of the Southern Uplands, consisting of Ordovician and Silurian rocks, into which are intruded the granitic and dioritic masses of Loch Doon, Cairnsmore of Carsphairn, Cairnsmore of Fleet and Spango. To the north lie Carboniferous rocks, including the Plateau lavas of the Renfrewshire-Ayrshire hills and, near Kilmarnock, the Millstone Grit Lavas.

## (ii) *Drift*

(a) *Glacial*. Over a large part of the area the solid rocks are concealed under a thick deposit of drift, boulder clay, often in the form of drumlins, predominating. The colour and composition of the boulder clay varies according to the district in which it occurs. Among Ordovician and Silurian rocks it is of a pale grey colour, on Old Red Sandstone it is red, and on the coalfields usually dark grey, blue or brown.

In the north and west the boulder clay is often shell-bearing and contains boulders of Highland origin. (Similar boulders are widespread north of the shell belt(18).) In the south and east, however, shells are uniformly absent, while the boulders and erratics have come from the Southern Uplands, those from the Loch Doon Granite being conspicuous. These facts, combined with the evidence obtained from the trend of striae and drumlins, indicate that the centre of Ayrshire was the meeting place of ice flowing southwards from the Highlands and ice flowing northwards from the Southern Uplands(2). The approximate line of junction passes near Girvan, Maybole and New Cumnock as shown in Fig. 1.

(b) *Recent*. Raised beaches, of various levels, are preserved along the coast, the 25-ft. raised beach being the best marked and most persistent. North of the mouth of the River Doon this beach forms a strip of flat land on which all the coast towns of the district are built. Throughout a great part of its course, however, its surface has been heightened and made uneven by inroads of blown sand. This is especially marked to the north of Ayr.

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River terraces are common along many of the larger valleys, good examples being found along the course of the River Ayr, from Stair to its mouth. Silted up lakes and bogs, occupied now by flat meadows or cultivated fields, are numerous between the Ayr and the Girvan valleys.

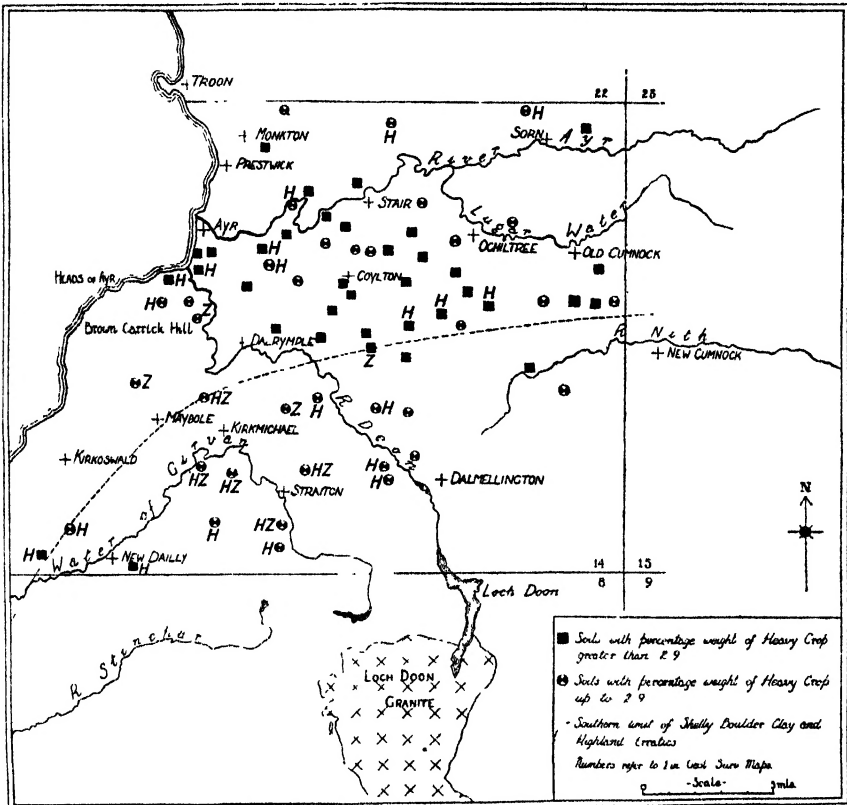


Fig. 1. Distribution of hypersthene (H), sphalerite (Z), and amount of heavy fraction in soils of South Ayrshire.

Many peat mosses, often of considerable extent, are found in the area, both on the low ground and among the hills. One of the largest is Aird's Moss, between the Lugar Water and the River Ayr. Other large areas of peat cover the high ground surrounding Dalmellington.

#### DESCRIPTION OF SOILS

In Table II the soils are grouped according to the underlying geological formations, with locality and a brief description. It will be noted that the majority of the soils are derived from glacial and recent deposits.

These soils were numbered by the Soil Survey members and are those on which they carried out a detailed chemical and physical examination. Jack<sup>(14)</sup>, in addition, gives a profile study with further chemical data, on certain soils within our area.

### MECHANICAL ANALYSES

In Table I are given a selection of results of the mechanical analyses, made according to the method of the Agricultural Education Association (A.E.A.)<sup>(1)</sup>, and carried out by members of the Chemistry Department, West of Scotland Agricultural College. These samples are representative of the soil textures as classified by them in the Soil Texture Map of the area published by the Geological Survey, 1932.

Table I

Group	...	1	2	3	3	4	5	6	7	8	9
Number	...	1032	789	882	267	2352	1315	2492	2177	905	2268
Coarse sand		14.25	15.6	71.3	1.3	10.8	17.0	14.6	28.95	40.6	10.65
Fine sand		40.35	43.5	11.7	4.3	29.25	21.65	36.3	37.9	30.1	22.2
Silt		13.3	21.6	5.0	18.4	16.8	22.68	15.0	11.3	8.7	19.2
Clay		17.0	10.6	5.6	51.0	22.8	22.95	24.0	13.9	11.4	19.3
Moisture		4.0	2.6	1.4	6.5	6.8	5.49	3.6	2.5	2.8	10.8
Loss of ignition		12.3	7.3	4.7	17.5	11.7	7.35	8.9	4.5	6.9	15.1
Total		101.2	101.2	99.7	99.0	98.15	97.12	102.4	99.05	100.5	97.25
Texture		Lm	Lm	Sa	C	Lb	Lb	La	Sb	Sb	C

The fine sand fraction used in the subsequent mineral analyses was obtained by an abbreviated mechanical analysis as outlined in the A.E.A. method, but without acid treatment.

### MINERALOGICAL ANALYSES

#### (i) *Heavy liquid separation*

The fine sand fraction was separated into three crops according to specific gravity, viz. (1) less than 2.63 (orthoclase crop); (2) 2.63–2.89 (quartz crop); (3) greater than 2.89 (ferro-magnesian crop). The method of separation is detailed by Hendrick and Newlands<sup>(12)</sup>, bromoform of s.g. 2.89 being the heavy liquid used.

The orthoclase crop, in addition to orthoclase, includes microcline, some plagioclase feldspars, rock fragments, plant remains and coally material. The high percentage of this crop recorded in certain soils is due to the presence of a large amount of rock fragments and carbonaceous material. As the name implies, quartz is by far the dominant mineral of the quartz crop. The heavier plagioclase feldspars are the only other

Table II

Soil	Locality	Group 1. Over Permian Sandstone: 1a on boulder clay.	Colour	Texture	Geology	Drift	Percentage of orthoclase (O), quartz (Q) and ferro-magnesian (F) crops in fine sand fraction		
							O	Q	F
1032*	Henryston, Stair	Dark brown		Lm	1a	N	12.4	82.4	5.2
1067	Lochhill, Stair	Brown		La	1a	N	2.8	94.9	2.3
1205	Parkhill, Tarbolton	Dark brown		Sb	1a	N	13.4	85.8	0.8
1206	Subsoil	Brown		La	1a	N	11.5	87.5	1.0
Group 2. Over barren Red Coal Measures: 2a on boulder clay, 2b on river terrace alluvium over boulder clay.									
134	Brocklehill, Ayr	Dark brown		Sb	2a	N	3.0	93.8	3.2
789	Glenconner, Ochiltree	Reddish brown		Lm	2a	N	1.3	94.8	3.9
1339	Thurpart, Auchinleck	Brown		La	2a	N	7.7	90.9	1.4
2300	Bonnyton, Ochiltree	Medium brown		Lb	2a	N	6.2	88.8	5.0
2355	Auchincloigh, Ochiltree	Grey-brown		Lm	2a	S	12.9	86.1	1.0
815	Burnton, Ochiltree	Brown		La	2b	N	18.4	70.8	10.8
Group 3. Over productive Coal Measures: 3a on boulder clay, 3b on fresh-water alluvium over boulder clay, 3c on fresh-water alluvium, 3d on raised beach.									
83	Broadhead, Coylton	Brown		C	3a	N	7.5	84.0	8.5
428	Duchvay, Coylton	Pale brown		Lb	3a	N	4.5	93.3	2.5
476	Bridgend, Coylton	Light brown		Lm	3a	N	2.4	96.2	1.4
481	Schav, Stair	Brown		La	3a	N	2.4	92.0	5.6
1180	Auchincruive, Ayr	Brown		La	3a	N	15.1	83.1	1.8
1181	Subsoil	Brown		La	3a	N	26.0	71.7	2.3
1184	Blackdyke, Sorn	Dark grey-brown		Lm	3a	N	18.6	80.2	1.2
1185	Subsoil	Brown		Lb	3a	N	11.4	87.7	0.9
1297	Raithhill, Coylton	Brown		Lb	3a	N	18.1	78.9	3.0
1298	Subsoil	Brown		Lb	3a	N	22.5	74.6	2.9
3053	Munnirey, Dalmellington	Dark brown		C	3a	S	12.5	86.4	1.1
267	Gallowhill, Coylton	Dark brown		C	3b	N	40.6	58.4	1.0
614	Treesmax, Stair	Reddish brown		Lb	3b	N	2.1	91.0	6.9

\* These are the serial numbers of the soils in the collection of the Department of Chemistry of the West of Scotland Agricultural College.

Sa = light sand; Sb = heavy sand; La = light loam; Lm = medium loam; Lb = heavy loam; C = clay.

1820	Darntaggart, Ochiltree	Brown	Sa	3b	N	8-2	84-5	7-3
2334	Laurshill, Ochiltree	Greyish brown	C	3b	N	30-6	66-2	3-2
88	Broadhead, Coylton	Brown	Lb	3c	N	3-85	91-5	4-65
154	Gadgirth Mains, Coylton	Rich dark brown	Sb	3c	N	4-7	92-3	3-0
882	Seafeld, Ayr	Dark brown	Sa	3d	N	1-4	88-2	10-4
884	Golfcourse, Ayr	Dark reddish brown	Sa	3d	N	2-3	89-4	8-3
885	Subsoil	Dark reddish brown	Sa	3d	N	3-0	89-1	7-9
907	Belleisle Golfcourse, Ayr	Brown	Sa	3d	N	2-6	80-7	16-7
1214	Aitkenbrae, Monkton	Dark brown	Sb	3d	N	5-5	91-8	2-7
1215	Subsoil	Dark brown	Sb	3d	N	4-8	90-4	4-8
Group 4. Over Millstone Grit : 4a on boulder clay, 4b on fresh-water alluvium.								
238	Bogside, Coylton	Brown	La	4a	N	10-2	86-8	3-0
333	Pettoch, Coylton	Medium brown	La	4a	N	6-9	87-2	5-9
2352	Burside, Old Cumnock	Dark brown	Lb	4a	N	13-5	81-7	4-8
2379	Over Glaisnock, Old Cumnock	Very dark brown	Sb	4a	N	12-1	81-9	6-0
2779	Waterhead, New Cumnock	Very dark brown	Lb	4a	S	6-6	88-6	4-8
3023	Moor near Lethanhill, Dalmellington	Dark brown	Lb	4a	S	10-4	87-9	1-7
2138	Barshare, Old Cumnock	Brown	La	4b	N	17-5	78-9	3-6
Group 5. Over Carboniferous Limestone Series : 5a on boulder clay, 5b on sandstone of Carboniferous Limestone Series.								
98	Whitefordhill, Ayr	Light brown	La	5a	N	5-0	91-2	3-8
966	Sandhill, Coylton	Light reddish brown	Lb	5a	N	6-9	89-1	4-0
968	Sandhill, Coylton	Light brown	C	5a	N	8-3	88-1	3-6
1223	Daldilling, Sorn	Dark brown	La	5a	N	8-2	88-4	3-4
1315	High Craighead, Kirkoswald	Brown	Lb	5a	S	8-5	90-0	1-5
1316	Subsoil	Yellowish brown	C	5a	S	7-1	89-2	3-7
1974	Forland, Old Cumnock	Light brown	La	5a	N	11-1	86-8	2-1
3014	Patna Hill, Straiton	Very dark brown	Lm	5b	S	24-5	73-8	1-7
Group 6. Over Calcareous Sandstone Series : 6a on boulder clay, 6b on fresh-water alluvium over boulder clay.								
38	Macnairston, Ayr	Dark brown	Lm	6a	N	8-2	88-9	2-9
1317	Balcarnie, Dailly	Dark brown	Sb	6a	S	18-0	74-2	7-8
1318	Subsoil	Brown mottled	Sb	6a	S	4-0	93-4	2-6
2492	Milreoch, Dalrymple	Reddish brown	La	6a	N	12-5	83-7	3-8
2498	Sandhill, Coylton	Reddish brown	Lm	6a	N	9-3	87-1	3-6
2928	Tranew, Kirkmichael	Reddish brown	Sb	6a	S	6-4	92-8	0-8
2949	Macnairston, Kirkmichael	Reddish brown	Sb	6a	S	8-3	90-6	1-1
937	Whitehill, Ayr	Pale brown	Lb	6b	N	48-9	47-9	3-2



Table II (continued.)

Percentage of orthoclase (O), quartz (Q) and ferro-magnesian (F) crops in fine sand fraction								
Soil	Locality	Colour	Texture	Geology	Drift	F		
						O	Q	F
Group 7. Over Old Red Sandstone: 7a on boulder clay over Upper Old Red Sandstone, 7b on peat over boulder clay, 7c on raised beach, 7d on boulder clay over Lower Old Red Sandstone.								
2177	East Mains of Newark, Maybole	Brown	Sb	7a	N	11.3	82.6	1.1
2904	Dalvennan, Kirkmichael	Yellow-brown	La	7a	S	10.1	87.2	2.7
3065	Shaws Knowes, Kirkmichael	Deep brown	La	7a	S	10.7	88.5	0.8
2601	Moor near Straiton	Dark red-brown	Sb	7b	S	7.6	91.7	0.7
851	Greenan, Maybole	Dark red-brown	Sb	7c	N	5.1	91.2	3.7
1326	South Thrieve, Kirkoswald	Dark brown	La	7d	S	3.9	94.5	1.6
1327	Subsoil	Mottled purplish	Lb	7d	S	2.5	95.0	2.5
2891	Blairbowie, Kirkmichael	Brown	La	7d	S	9.4	87.8	2.8
2976	Meadownay, Maybole	Brown	La	7d	S	17.4	81.0	1.6
Group 8. Over Igneous Rocks: 8a on boulder clay over Lower Old Red Sandstone lavas, 8b on boulder clay over Teschenite intrusions, 8c on raised beach over Millstone Grit lavas, 8d on alluvium over Lower Old Red Sandstone lavas, 8e on Permian lavas, 8f on Millstone Grit lavas, 8g on Lower Old Red Sandstone lavas, 8h on Teschenite intrusions.								
2659	Knockburnie, New Cumnock	Dark red-brown	Lb	8a	S	19.9	78.5	1.6
2540	Knockshinnoch, Dalrymple	Brown	La	8b	N	7.7	88.2	4.1
2738	Rankinston, Coyton	Dark brown	Lb	8b	S	6.3	88.6	5.1
3035	Grimmet, Straiton	Dark brown	Lb	8b	S	23.5	75.7	0.8
3048	Moor near Rankinston, Dalmellington	Dark brown	C	8b	S	27.5	70.0	2.5
858	Greenan, Maybole	Rich dark brown	Sa	8c	N	1.3	90.7	8.0
905	Belleisle Golfcourse, Ayr	Brown	Sb	8c	N	4.9	89.3	5.8
906	Subsoil	Brown	La	8c	N	5.7	88.3	6.0
2166	Balig, Maybole	Brown	La	8d	N	16.7	81.3	2.0
2922	Craigbrae, Straiton	Dark brown	La	8d	S	17.8	80.4	1.8
3094	Dalmorton, Straiton	Rich dark brown	La	8d	S	13.2	85.0	1.8
638	Hunterston, Stair	Dark reddish brown	Lm	8e	N	2.6	93.0	4.4
727	East Tarelgin, Ochiltree	Dark sepia-brown	Lb	8e	N	4.4	89.6	6.0
731	East Tarelgin, Ochiltree	Dark red-brown	Lb	8e	N	4.3	88.0	7.7
1118	Watson, Ochiltree	Brown	La	8e	N	1.5	95.6	2.9
54	Lochfergus, Ayr	Dark brown	Lb	8f	N	25.6	72.2	2.2
61	Lochfergus, Ayr	Dark brown	Lm	8f	N	10.6	88.4	1.0
2268	Auchenroy, Straiton	Deep brown	C	8g	S	37.0	62.0	1.0
2437	Blairston Mains, Maybole	Red-brown	Lm	8g	N	15.3	82.4	2.3
2275	Southcraig, Coyton	Light red-brown	Lm	8h	N	10.4	85.6	4.0

minerals recorded in this separation. The bulk of the mineralogical examination was carried out on the ferro-magnesian crop which had the greatest mineral variation.

Hart (11) briefly enumerates the causes of variation of ferro-magnesian percentages within soils of the same group, and they can be applied to this area. He states: "...it is only to be expected that there are local concentrates in the drift deposits as in sediments. Also the varying textures of the glacial drifts... affect the rate of solution of the minerals. Different degrees of cultivation may also have some effect." Making allowances for such variations it is found that soils derived from the Northern Drift have a higher ferro-magnesian percentage than those obtained from the Southern Drift. The average percentage weight of ferro-magnesian minerals from the former is 4.6, while from the latter it is 2.1. At the junction of the two drifts there is a certain amount of overlap. A group of soils showing a low ferro-magnesian percentage to the north-west of Coylton is probably influenced by the Permian and the Barren Red Measures. Boyle (5), investigating the former in the Mauchline Basin, and Smellie (19) the Upper Barren Red Measures to the east of Glasgow, record a low percentage of heavy minerals.

Soils over raised-beach material and over fresh-water alluvium show a higher ferro-magnesian percentage than those over boulder clay. Generally speaking, however, the mineral content of these post-Glacial deposits is similar to that of the boulder clay, and indicates that the former are merely resorted boulder clay material.

By means of the triangular graphs (Fig. 2) comparison has been made of our results with those obtained by Hart in south-east Scotland and by Hendrick and Newlands (12, 13) in north-east Scotland. It will be observed that our results compare more favourably with those of Hart (10, 11), as is to be expected from the geological similarity of the areas.

### (ii) *Mineral description*

*Quartz* is by far the commonest mineral and constitutes the major proportion of the soil. The grains are fresh, subangular to rounded and usually contain numerous large and small fluid and mineral inclusions, the latter often giving a dusky appearance to the grain.

*Felspars* are next in order of abundance to quartz. They differ from that mineral in their platy habit and greater tendency towards decomposition.

Both fresh and decomposed orthoclase grains are present, generally subangular and with numerous inclusions. By far the majority of the plagioclase felspars are decomposed and rounded. Positive identification of any individual member of the series was therefore difficult. Some grains of labradorite, however, were identified in

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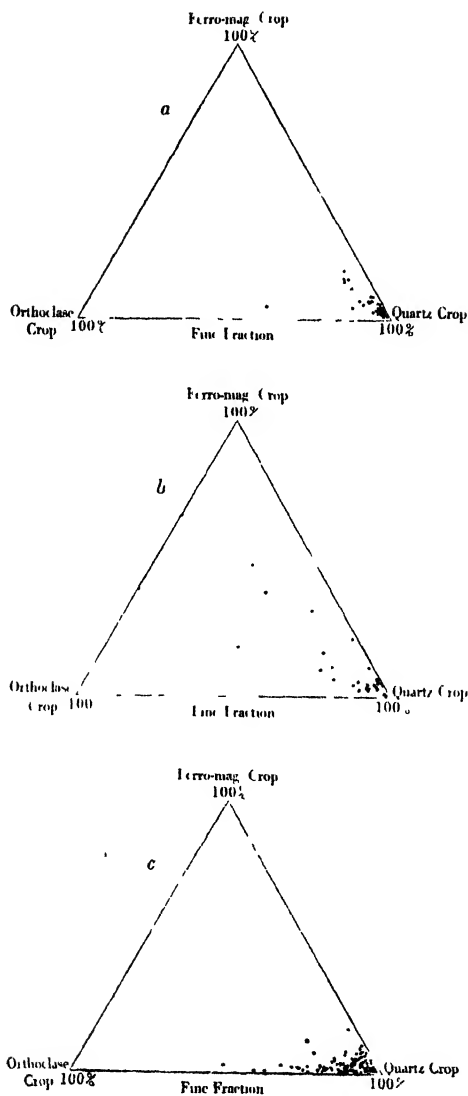


Fig. 2. Graphs of crop percentages of fine fraction.

- (a) Hart —All soils from S.E. Scotland. (b) Hendrick and Newlands —23 soils from N.E. Scotland, 2 soils from E. Scotland, 1 soil from Ayrshire, 4 soils from England. (c) All soils from South Ayrshire.

the quartz crop. Microcline was always fairly fresh, angular to subangular, of large grain size and showing the characteristic cross-hatched twinning.

*Minerals of specific gravity greater than 2.89, in alphabetical order*

*Apatite* is generally scarce, though common in a few soils. It occurs as colourless, rounded (egg-shaped), subangular and angular prismatic grains. Most contain numerous small black inclusions, while a few of the prismatic form show a central pleochroic core. Tyrrell<sup>(20)</sup> has recorded an abundance of this mineral in the igneous rocks of the area.

*Augite* occurs in all soils, of large grain size and with a characteristic angular to subangular shape. With iron oxides it is the dominant heavy mineral in the majority of the soils. Light brownish green and green grains are commonest, though colourless and purple varieties are plentiful. The former show greater decomposition, especially along cleavages, than the latter, which are characterised by fresh appearance, lack of cleavage traces and scarcity of inclusions. The intermediate coloured varieties contain colourless, brown and black inclusions of variable size, sometimes giving the grain a dusky appearance. Those of darker colour often show pleochroism.

Great difficulty was found in differentiating between undoubted augite and diopside. A few fresh pale green grains were definitely identified as the latter mineral.

*Barkevikite* is found in the majority of the soils as common to scarce, though in two cases it is the dominant ferro-magnesian mineral. Grains are generally fresh, angular to subangular, of deep brown colour and exhibiting the characteristic deep brown to yellow-brown pleochroism. Black and colourless inclusions were noted.

*Biotite* is also of widespread occurrence, though never abundant in any soil sample. It is found as subangular to rounded platy, brown leached flakes. Inclusions are numerous, around some of which well-marked pleochroic haloes were observed.

*Chloritic material.* Under this heading is included chlorite proper and all those indeterminate decomposition products of ferro-magnesian minerals. It is usually common to all soils. Chlorite itself is found as dark green micaceous-like flakes and is isotropic in almost every case. Pleochroic grains are negligible. Some of the decomposition material is probably serpentinised olivine, since as Tyrrell<sup>(21)</sup> points out the olivine of the igneous rocks of the area is very susceptible to alteration.

*Epidote* occurs as subangular, lime-green, pleochroic grains, sporadic in distribution and rare in those soils in which it was identified.

*Garnet* is found in almost every soil and is in some of them a very common mineral. The grains show all degrees of shape, size and angularity, but the cleaved (dodecahedral), fractured grain is a characteristic of the soils. Colourless, pink and green varieties were observed, the colourless being most common. Pink grains exhibit cleavage infrequently, while green garnets are rare and uncleaved. Little decomposition was noted. Large brown and small black inclusions were observed, the latter, in some cases, giving the grain a dusky appearance (Fig. 3).

*Glaucophane.* In a few soils, blue grains, pleochroic to yellow-green, were identified as glaucophane. This mineral has been recorded by Mackie from rocks in Ayrshire (17).

*Hornblende* is present in almost all the soils examined, in varying amounts from rare to dominant. The dominance of this mineral is more pronounced in the soils from the Southern Drift. Most grains are angular prismatic with frayed edges and showing one good cleavage, though subangular tabular grains with the characteristic horn-

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blende cleavages were also observed. The colour ranges from light green to brownish green. It was noted that the hornblende from the Southern Drift was fresher in appearance than that from the Northern Drift, which showed incipient alteration and was more often of the brownish green variety. Large and small black and colourless inclusions are common to all types and are occasionally arranged in definite manner, e.g. along cleavage planes.

*Hypersthene* is found in the majority of the soils from the Southern Drift but is scarce in those from the North. Both fresh and decomposed tabular and prismatic

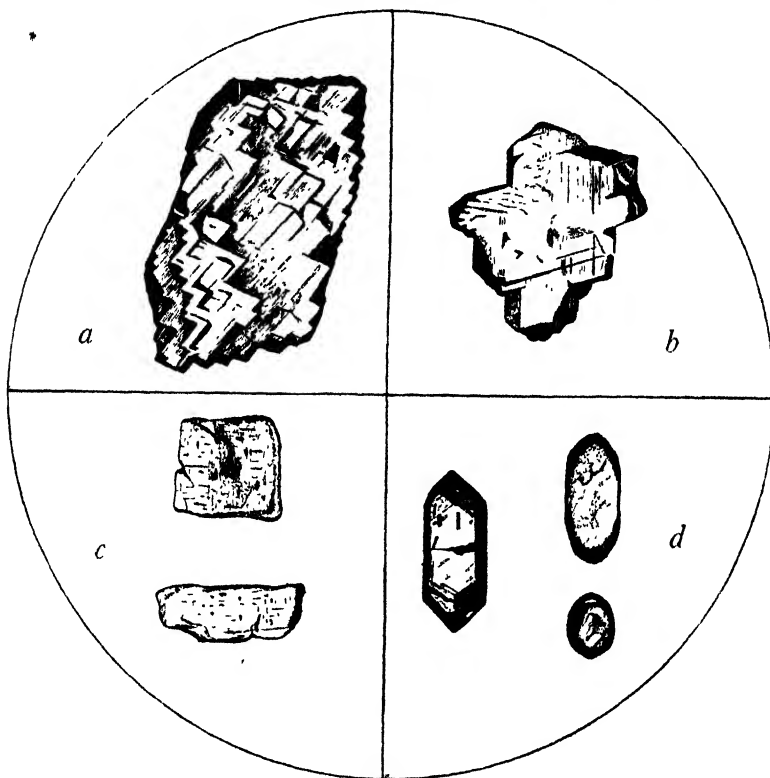


Fig. 3. *a*, Sphalerite.  $\times 200$ . *b*, Garnet.  $\times 150$ . *c*, Hypersthene.  $\times 150$ . *d*, Zircon.  $\times 150$ .

subangular grains, showing the characteristic green to pink pleochroism, were identified. Black inclusions, often arranged along cleavages, are common (Fig. 3).

A few grains resembling hypersthene but non-pleochroic and colourless to pale green were recorded as *Enstatite*.

*Iron oxides.* Those identified were, in order of abundance, haematite, limonite, ilmenite and leucoxene, and magnetite. This group predominates in the ferro-magnesian crop. Grains are of large size; those of haematite and limonite are of earthy form and usually rounded, while those of magnetite and ilmenite are crystalline and show more angularity.

*Muscovite* is absent from very few of the soils, and ranges from rare to common. It occurs as rounded, colourless plates of large grain size. Inclusions, among which were noted small zircons and rutiles, were frequently observed, though some grains were exceptionally free from them. Some of the former may be leached biotite.

*Rutile* is found in the majority of the soils as subangular fragments and prismatic grains, which occasionally show well-defined pyramidal termination. The colours vary from deep red to yellow, the latter being fresher than other varieties. Diagonal striations, geniculate twinning and sagenite (lattice) forms were observed in a few cases.

*Sphalerite*. A brown, translucent mineral with adamantine lustre and well-defined dodecahedral cleavage was identified as sphalerite (zincblende). This was confirmed by comparing with undoubted crushed material. It occurs as large angular isotropic fragments, containing few inclusions. It was found in nine soils which were practically all restricted to the Southern Drift (Fig. 1).

*Sphene* (titanite), though present in many soils, is usually rare in individual soil samples. The mineral is generally fresh, brown to colourless, shows slight pleochroism, occasional twinning and often lack of extinction. Mostly angular in habit and shows little trace of cleavage. Inclusions are scarce. In many cases positive diagnosis was found to be rather difficult.

*Staurolite* is a rare mineral in the soils of this area, usually showing decomposition, numerous inclusions, irregular subangular shape and platy habit. Grains identified exhibit the golden yellow colour and typical pleochroism.

*Tourmaline* is widespread in occurrence and varies in quantity in individual soils. It is the most variable mineral found both in shape and colour. Slender prismatic and stumpy grains exhibiting good crystal terminations and polar structure are frequent. Angular to subangular and rounded fragments are also present. The colours noted are black, brown (commonest), purple, blue, green and yellow. Pleochroism is intense and common to all grains except basal sections, a few of which were observed. Particoloured polar forms occur. Inclusions, where abundant, give a dusky appearance to grains. Vertical striations and a basal parting were observed in a few cases.

*Zircon* is a common mineral throughout the area, being absent from only two of the soils. Round, egg-shaped and well-defined prismatic forms are typical. Perfect cubedra, often zoned, were frequently observed. The majority are colourless, but yellow and purple varieties are also present in fair amount. Colourless and black inclusions, the latter occasionally giving the grains a dusky appearance, occur abundantly (Fig. 3).

#### *Minerals of rare occurrence*

Anatase, ? brookite, aragonite, calcite, corundum, fluor spar, kyanite, olivine and serpentine, ? monazite, picotite, ? zoisite.

### ANALYSIS OF MINERAL DISTRIBUTION

A. Geikie, in the Geological Survey Explanation of Sheet 14, points out that the composition and colour of the boulder clay varies according to the formation upon which it rests. We have not been able to detect a similar correspondence in regard to the distribution of the heavy minerals

Table III. *Table of mineral frequencies, omitting quartz and felspars*

	Group 1										Group 2										Group 3									
Apatite	1032	N	R	D	R	R	R	R	R	R	1206	N	R	D	R	R	R	R	R	R	83	N	D	D	R	R	R	R	R	R
Augite	1067	N	R	D	R	R	R	R	R	R	1205	N	R	D	R	R	R	R	R	R	428	N	D	D	R	R	R	R	R	R
Barkevikite			R	D	R	R	R	R	R	R			R	D	R	R	R	R	R	R	476	N	D	D	R	R	R	R	R	R
Biotite			R	D	R	R	R	R	R	R			R	D	R	R	R	R	R	R	481	N	R	D	R	R	R	R	R	R
Chloritic Mat.			R	D	R	R	R	R	R	R			R	D	R	R	R	R	R	R	1180	N	R	D	R	R	R	R	R	R
Epidote			R	D	R	R	R	R	R	R			R	D	R	R	R	R	R	R	1181	N	R	D	R	R	R	R	R	R
Garnet			R	D	R	R	R	R	R	R			R	D	R	R	R	R	R	R	1184	N	R	D	R	R	R	R	R	R
Glaucophane			R	D	R	R	R	R	R	R			R	D	R	R	R	R	R	R	1185	N	R	D	R	R	R	R	R	R
Hornblende			R	D	R	R	R	R	R	R			R	D	R	R	R	R	R	R	1297	N	R	D	R	R	R	R	R	R
Hyperssthene			R	D	R	R	R	R	R	R			R	D	R	R	R	R	R	R	1298	N	R	D	R	R	R	R	R	R
Iron oxides			R	D	R	R	R	R	R	R			R	D	R	R	R	R	R	R	3053	S	R	D	R	R	R	R	R	R
Muscovite			R	D	R	R	R	R	R	R			R	D	R	R	R	R	R	R	3053	S	R	D	R	R	R	R	R	R
Rutile			R	D	R	R	R	R	R	R			R	D	R	R	R	R	R	R	3053	S	R	D	R	R	R	R	R	R
Sphalerite			R	D	R	R	R	R	R	R			R	D	R	R	R	R	R	R	3053	S	R	D	R	R	R	R	R	R
Sphene			R	D	R	R	R	R	R	R			R	D	R	R	R	R	R	R	3053	S	R	D	R	R	R	R	R	R
Staurolite			R	D	R	R	R	R	R	R			R	D	R	R	R	R	R	R	3053	S	R	D	R	R	R	R	R	R
Tourmaline			R	D	R	R	R	R	R	R			R	D	R	R	R	R	R	R	3053	S	R	D	R	R	R	R	R	R
Zircon			R	D	R	R	R	R	R	R			R	D	R	R	R	R	R	R	3053	S	R	D	R	R	R	R	R	R

D=dominant; d=abundant; dr=common; r=rare; R=rare; N from area of Northern Drift (Fig. 6); S from area of Southern Drift (Fig. 6)

[illegible]



Table III (continued)

Group 8

2659	R	d	dr	—	dr	R	—	—	R	—	D	R	R	—	—	—	d
2540	—	D	r	R	r	—	—	—	R	—	D	r	r	dr	R	—	dr
2738	—	D	dr	r	r	—	—	—	R	—	d	R	r	—	R	?	r
3333	R	dr	R	R	r	—	R	—	D	R	D	—	r	—	R	—	dr
3303	R	D	r	R	dr	—	r	—	R	—	d	R	r	—	R	—	r
858	R	D	R	r	dr	—	d	—	dr	r	D	r	R	—	R	—	r
1506	R	D	R	R	r	R	d	—	r	—	D	r	R	—	—	R	r
906	R	D	r	R	r	—	dr	—	dr	R	d	r	r	—	R	—	r
1091	R	D	r	r	r	R	d	—	dr	R	D	dr	R	—	—	r	dr
2922	r	d	dr	—	r	—	dr	—	r	r	D	r	r	—	—	—	D
3045	dr	R	R	R	r	R	r	R	D	d	r	R	—	R	R	R	dr
333	R	D	r	R	r	—	r	—	r	—	D	r	r	—	R	—	r
727	dr	D	r	R	r	—	dr	R	r	—	d	r	dr	—	—	r	dr
711	—	D	R	—	r	—	r	—	—	D	r	—	—	—	R	R	
1118	R	D	r	R	r	?	dr	—	R	—	D	r	r	—	?	—	dr
145	R	D	r	R	r	—	r	—	r	—	D	—	R	—	r	R	r
19	R	D	r	—	r	—	d	—	dr	—	D	r	dr	—	R	—	d
2268	R	dr	R	—	—	—	r	—	d	R	D	—	R	—	R	dr	d
2437	R	d	r	R	R	?	r	—	R	—	D	R	r	dr	—	R	dr
2275	R	D	R	R	r	?	dr	—	dr	—	D	r	r	—	R	R	r

Apatite  
 Augite  
 Barkevikite  
 Biotite  
 Chloritic Mat.  
 Epidote  
 Garnet  
 Glaucophane  
 Hornblende  
 Hypersthene  
 Iron oxides  
 Muscovite  
 Rutile  
 Sphalerite  
 Sphene  
 Staurolite  
 Tourmaline  
 Zircon

of the soils. On the other hand, the distribution does conform, in some respects, with the division of the drifts into southern and northern areas, as indicated on Fig. 1.

The relation is most definite in the case of sphalerite. As recorded in Table III, seven out of twenty-five southern samples yielded this mineral,

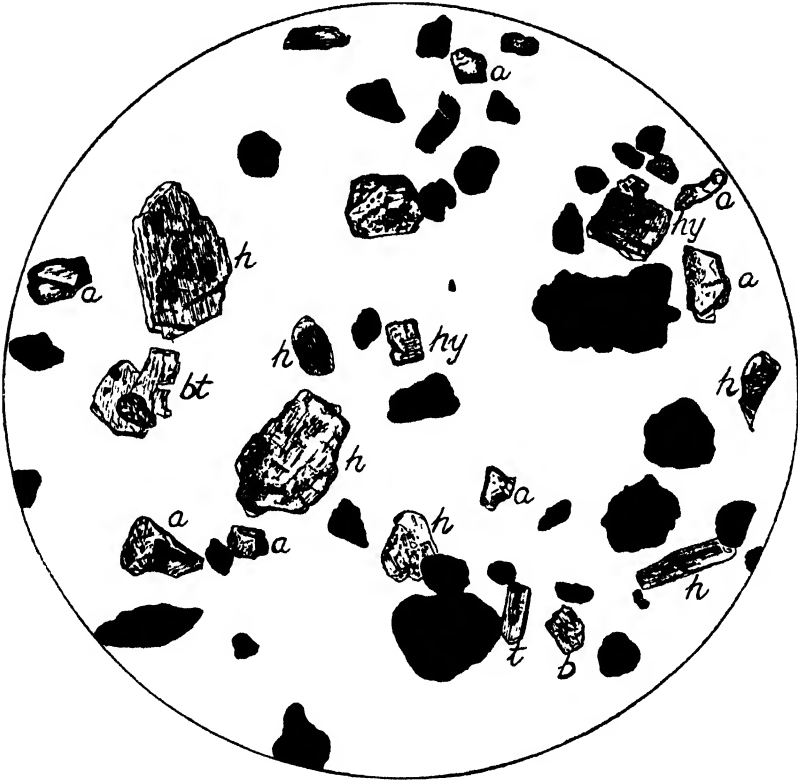


Fig. 4. Soil No. 1315. Heavy residue; magnetic crop.  $\times 50$ . *a*, Augite; *b*, Barkevikite; *bt*, Biotite; *h*, Hornblende; *hy*, Hypersthene; *t*, Tourmaline. Iron oxides black.

as against two out of fifty-nine of the northern samples. There can be little doubt that most of the sphalerite has come from mineral veins intruded into the Ordovician-Silurian belt of the Southern Uplands<sup>(23)</sup>.

Similar, though less striking, results have been found for hypersthene. In this case the numbers for the southern area are eighteen out of twenty-five, and for the northern area thirteen out of sixty. Probably the norite of the Loch Doon "Granite" complex<sup>(8)</sup> is responsible for most of this

## 18 Mineral Composition of the Soils of South Ayrshire

mineral. The much smaller Distinkhorn granite north of Sorn(15), and various outcrops of lavas of Lower Old Red Sandstone age, may have supplied some of the more northerly occurrences. It should, however, be remembered that there are various causes that tend to lead to an in-

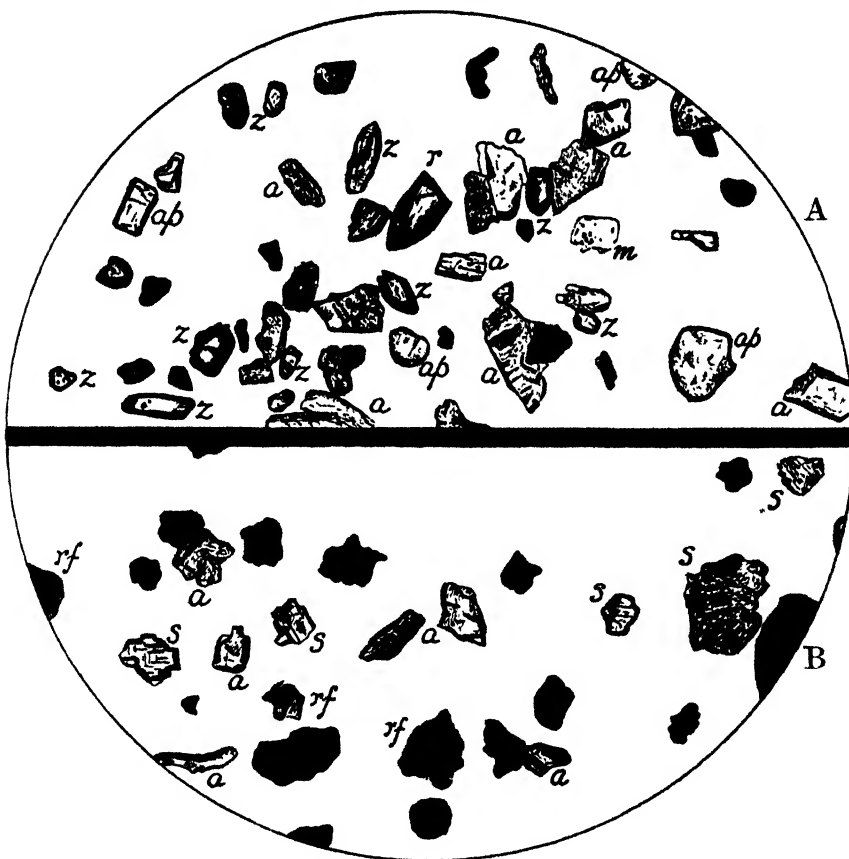


Fig. 5. A. Soil No. 1820. Heavy residue; non-magnetic crop.  $\times 50$ . B. Soil No. 2437. Heavy residue; magnetic crop.  $\times 50$ . *ap*, Apatite; *a*, Augite; *m*, Muscovite; *rf*, Rock fragments; *r*, Rutile; *s*, Sphalerite; *z*, Zircon. Iron oxides black.

definite result. Thus the position of the line drawn between southern and northern drifts is only an approximation, while the River Doon must have carried southern minerals northwards to the sea.

Fresh green hornblende is more abundant in the southern soils, while barkevikite is more pronounced in a belt of country just north of and parallel to the line of junction of the drifts. The abundance of the green hornblende to the south may be traced to the influence of the Loch Doon

"Granite" mass and the Southern Upland greywackes (16). Barkevikite, on the other hand, is derived from the alkaline intrusives so common in the central part of Sheet 14 (22, 6).

Augite, though by far the dominant ferro-magnesian mineral, is superseded to some extent in the south by green hornblende. This agrees with the distribution of the main augite-bearing igneous rocks, which are commoner to the north.

Other common minerals such as zircon, garnet, tourmaline, apatite and rutile have all been found in sediments of similar types to those of Ayrshire (3, 19). The assemblage described by Bosworth (4) from the carboniferous rocks of the Midland Valley of Scotland is very similar to that obtained in the soils.

A characteristic of the total assemblage is the freshness and angularity of the mineral grains. This is especially true of the ferro-magnesian minerals, for example augite, hornblende and barkevikite, which presumably have been derived from the igneous rocks of the county.

Our results agree closely with those obtained by Hart (10, 11) in south-east Scotland. The soils examined by Hart mostly came from the Midland Valley, and three comparable examples were described by Hendrick and Newlands (13). Augite, as might be expected from the abundance of basic lavas and intrusions in many parts of the Midland Valley, proves to be widespread as a soil constituent. On the other hand, the sphalerite and the frequent hypersthene of the southern part of Sheet 14 seem to be rather special features.

#### RELATION OF MINERALOGY TO PEDOLOGY

The complicated nature of the source of the parent material and of the mineral content of the boulder clay has always been a question of doubt and difficulty in the unravelling of the formation of a soil type. The present work was suggested as a means of determining the parent material of the soils, and, though not exhaustive, would seem to show that there is a method at hand for an initial classification of soils, such as is found by the authors in South Ayrshire, viz. a northern and southern drift type of soils. A preliminary investigation of several typical profiles along with numerous soils and subsoils, all from the same area, tends to confirm this conclusion.

The results obtained would seem to justify the adoption of mineralogical methods in the study of soils and in forming a basis on which to begin the study of the pedogenic processes involved.

## 20 *Mineral Composition of the Soils of South Ayrshire*

### SUMMARY

1. A detailed mineralogical analysis was carried out on soils, specially selected by members of the Soil Survey.
2. The heavy liquid separation showed a higher percentage of ferro-magnesian minerals in soils from the Northern than the Southern Drift area.
3. The minerals identified in the soils may be referred to fairly local origins, but no close relationship with subjacent rock has been established.
4. The mineral assemblage was characteristically fresh and angular.
5. Hypersthene, sphalerite and hornblende are characteristic of the Southern Drift, while barkevikite is more pronounced in the Northern Drift.
6. Comparisons are made with results obtained by investigators in other areas.

In conclusion the authors wish to express their gratitude to Prof. E. B. Bailey, Prof. D. N. McArthur, Dr C. L. Whittles, to the staff of the Geology Department, Glasgow University, and to those of the Chemistry Department, West of Scotland Agricultural College, Glasgow, for advice, encouragement and practical assistance, always freely given.

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## MAPS REFERRED TO IN TEXT

Geol. Surv. Scot. 1907,  $\frac{1}{4}$  in. to 1 m. Geol. Map, Sheet 16.

Geol. Surv. Scot. 1932, 1 in. to 1 m. Soil Texture Map, Sheet 14, Ayr.

Geol. Surv. Scot. 1933, 1 in. to 1 m. Geol. Map, Sheet 14, Ayr (solid and drift).

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# STUDIES ON THE RELATION BETWEEN CULTIVATION IMPLEMENTS, SOIL STRUCTURE AND THE CROP

## I. SOME PRELIMINARY OBSERVATIONS ON THE MEASUREMENT OF SOIL STRUCTURE, WITH A DESCRIPTION OF AN INSTRUMENT FOR THE MEASUREMENT OF SOIL RESISTANCE

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*(With Plate I and Four Text-figures)*

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### I. INTRODUCTION

THE testing and the ordinary farming use of cultivation implements are still largely governed by empirical principles. The design and construction of cultivation implements have reached a high standard as the result of centuries of accumulated practical experience in soil cultivation. But no material advances have been made in the methods of designing and testing those parts of the implements which are intimately concerned with the working of the soil. The designing of these parts is to-day almost as much a matter of trial and error as it was a century ago, and experiments on cultivation implements are frequently valueless because only the end-effects produced on the crop are recorded, no attempt being made to study how effects on the crop are produced by differences in the soil. If the design and testing of cultivation implements are to be substantially raised above their present level, the precise actions of implements on the soil must be discovered. The real criterion of implement effects on the soil lies in the consequential effects on the crops,

and to understand how cultivations affect final yield, it is essential to know how they influence the successive stages of growth by which, of course, yield is determined. Thus, developmental studies of the crops, coupled with physical observations on the soil, should form the basis of investigations on cultivation implements.

Little of a quantitative nature is known concerning the relationship between soil structure and plant growth. The chief reason for this lack of knowledge has been the almost complete absence of any recognised measurements by means of which soil structure could be defined. No progress was possible until attempts were made to employ metrical observations in defining soil structure. During recent years, certain metrical methods have been developed. These methods are themselves mostly of an empirical nature and none gives an entirely satisfactory picture of soil structure, but until some better technique is developed, they provide means by which the relation between implements, soil structure and plant growth may be surveyed. The following are among the more useful of these methods:

(1) Measurement of the resistance to the passage through the soil of various implements or special probes.

(2) Sieving tests to determine the proportions of aggregates of different sizes (Keen<sup>(1)</sup>).

(3) Fixation of the soil by Pigulevski's method (Miscenko<sup>(2)</sup>).

Studies on the rate of percolation of water through the soil may in some circumstances throw additional light on soil structure, but a satisfactory technique for the use of this test under field conditions has yet to be developed. The three methods enumerated above may be used in the field to obtain some idea of soil structure, but they are all dependent on more fundamental quantities. For this reason, it is essential that the conditions under which such measurements are made should be defined, and of these conditions, moisture content of the soil is of outstanding importance.

The present paper contains a short discussion of the nature and effects of soil compactness and resistance, together with a description of an instrument for the measurement of soil resistance. The application of this apparatus and of certain other methods for the study of soil structure to experiments on the "Gyrotiller"<sup>1</sup> is briefly described.

<sup>1</sup> The "Gyrotiller" is a Diesel-engined rotary cultivator, manufactured by John Fowler and Co., Ltd., Leeds.



## II. DISCUSSION: THE NATURE AND MEASUREMENT OF SOIL CONSOLIDATION

Soil consolidation is a complex characteristic. Its nature is imperfectly understood and little is known either concerning methods of producing consolidation or of the effects of consolidation on plant growth. Soil "compactness" is one of many factors which influence consolidation. In pure dry sand, compactness may be synonymous with consolidation, but in agricultural soils it is not. It is possible to treat similar soils in such a way that the more compact one may be the less consolidated. The distinction between compactness and consolidation is illustrated by the experiments described in section V of this paper. A close relationship exists between soil consolidation and the resistance presented by the soil to the passage of cultivation implements or to the slow penetration of metal probes. The resistance of the soil to penetration by implements or probes gives a measure of the state of consolidation. Keen and Cashen (3), Davies (4) and Heath (5) have made use of this relationship in various ways to obtain a measure of soil consolidation. The apparatus of Keen and Cashen and of Heath consists of a weight falling through a constant distance and forcing a metal point into the soil in the manner of a pile-driver. The amount of penetration for each impact is measured on a scale, and the number of impacts per unit of penetration gives a measure of soil consolidation at various depths. Davies' "Compactometer" is an instrument which gives an automatic record of the force required at various depths to push a steel probe into the soil. The force is measured by the extension of springs connecting the handle and the probe, and is recorded as a depth-consolidation curve.

These methods give measurements of the resistance of the soil to the penetration of a steel point at various depths. This resistance, like the resistance of the soil to the passage of cultivation implements, is the integrated effect of several physical factors. The chief of these factors are:

- (1) *Soil cohesion.* Forces are exerted when soil particles are torn apart.
- (2) *Soil plasticity.* Work is done against the internal friction of the soil during its deformation round the probe.
- (3) *Surface friction* between the soil and the metal.

Since neither cohesion nor plasticity is a clear-cut physical factor, it cannot be expected that soil-resistance measurements will bear a simple relationship to other measurements of soil structure. They may, however,

if interpreted carefully, be useful in comparative studies of the relation between soil structure and plant growth. Eden and Maskell<sup>(6)</sup> have shown that a correlation may exist between the resistance of the soil to the passage of a plough, and plant growth and development at various places over a field. Similar correlations between soil resistance and plant growth are demonstrated in section V, where major differences in resistance are artificial ones produced by the cultural operation of gyro-tilling in contradistinction to the natural differences investigated by Eden and Maskell.

### III. THE MEASUREMENT OF SOIL RESISTANCE: DESCRIPTION OF APPARATUS

The pile-driving method employed by Keen and Cashen and by Heath is slow and laborious. Heath<sup>(5)</sup> relates that in very hard dry soil, one determination down to 35 cm. depth may take half an hour. Speed of working is an important consideration in the measurement of soil resistance, for a shower may have a great effect on the results obtained. Moreover, it is essential to obtain a fairly large number of samples. Davies' method is rapid, but the speed of pushing the probe into the soil may vary between wide limits, giving an erratic action. Though the effects of variations of speed within narrow limits are negligible, wide variations of speed may alter the resistance to penetration considerably. Davies' instrument is also expensive.

After preliminary tests involving the use of impacts and of dead weights, the apparatus described below, which entails the use of springs for forcing a probe into the soil, was designed.

A steel probe, *E* (Text-fig. 1), is forced into the soil through two springs, *C*. One end of each spring is attached to the top of the probe through a rocker arm, *D*, and the other is attached to a frame, *B*, which is driven downwards along guide rods, *F*, through screw and bevel gearing, *A*, by turning a handle. The resistance to penetration of the probe at any depth down to 15 in. is measured by the extension of the springs and is automatically recorded as a depth-resistance-curve. Recording is effected by a stylus, *S*, which is fixed to the top of the probe. It writes on a drum, *G*, mounted on the frame, *B*. The drum is of the engine-indicator type. It is driven as follows: A piece of indicator cord, *K*, is attached to the fixed part at *H* (Text-fig. 1 *a*). The cord passes round a small idler pulley, mounted on a horizontal axis, on to a large pulley, *L*, whose axis is vertical. The cord is fixed to this pulley, and as the frame, *B*, moves downwards, the pulley is driven as the cord is

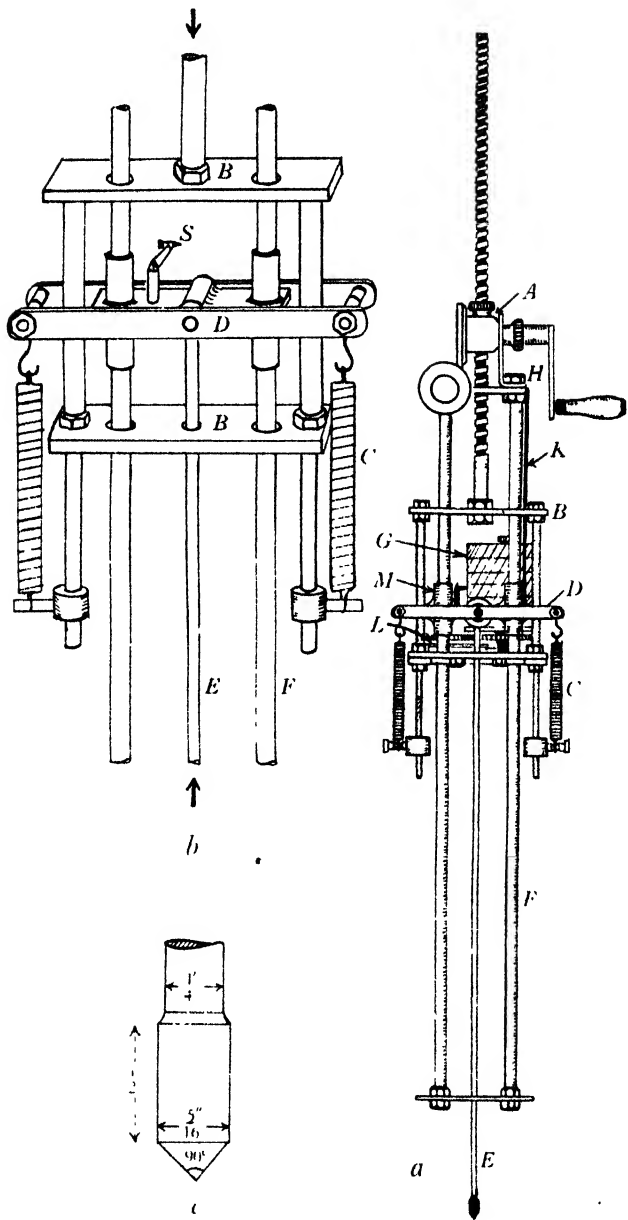


Fig. 1. Diagrams illustrating construction of apparatus used for the measurement of soil resistance.

unwound from it. The peripheral speed of the drum is adjusted to be exactly half the linear speed of the screw by having the pulley and drum of equal diameters and driving the drum from the pulley through half-time gear wheels. When the frame is moving downwards, the drum is driven against the force of a spring mounted inside it. On the return, the spring drives the drum, returning it to its original position.

It should be observed that the drum is driven directly from the screw, and distances of  $\frac{1}{2}$  in. along the horizontal axis of the chart attached to the drum correspond accurately to intervals of 1 in. in the downward motion of the screw. But the downward movement of the probe is governed partly by the resistance that it meets. This may be illustrated as follows: Suppose  $P$  (Text-fig. 2) is any point on the curve

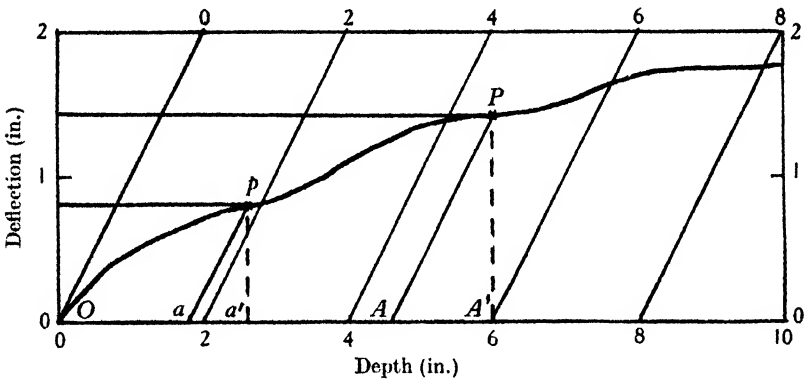


Fig. 2. Diagram illustrating the use of an inclined axis.

described by the stylus. It is vertically above  $A'$  and  $PA'$  is the extent to which the springs have been stretched. The point of the probe has therefore not entered the ground a distance  $OA'$ , but has penetrated a distance  $(OA' \text{ minus } PA')$ . And since the scale along the abscissa is half that along the ordinate, the true depth at which the resistance is  $PA'$  is given by  $OA$ , where  $AA'$  is equal to  $\frac{1}{2}PA'$ . Similarly, for point  $p$ , the corresponding depth is given by  $Oa$  and not by  $Oa'$ . In order to facilitate the derivation of results from the curves, a special chart with the axis inclined at the requisite angle is used.

The probe which has been most used (Text-fig. 1c) consists of a  $\frac{1}{4}$  in. diameter rod of spring steel, with a  $\frac{5}{16}$  in. diameter case-hardened point screwed into its lower end. The angle of the point itself is  $90^\circ$ . Material which flows past the point is therefore bent at the point through  $45^\circ$ , the natural shear angle for sand and some other materials. A straight-sided

probe of  $\frac{5}{16}$  in. diameter with a  $90^\circ$  point may also be used. The upper end of the probe is screwed into a rectangular piece of mild steel, to which are welded two tubes, *M* (Text-fig. 1 *a*). These tubes slide along the two stationary rods which connect the base-plate and the gearbox at the top. The rocker arm, *D*, pivots about its centre, directly above the top of the probe. Other details of construction are illustrated in Text-fig. 1 and in Pl. I.

#### IV. USE OF THE SOIL-RESISTANCE APPARATUS

The apparatus may be calibrated by recording the deflections produced by varying forces applied to the point of the probe. A convenient method is to press the point of the probe on to the platform of a barn weighing machine and record on the drum the deflections obtained when the force exerted through the probe just balances the weight on the machine. A series of deflection-resistance readings is thus obtained, and if these are plotted on a graph, the calibration curve is found to be a straight line, which passes through the origin when the attachment of the springs is correctly adjusted. Deflections obtained experimentally may then be easily converted into the corresponding resistances. Four different pairs of springs have been used to suit varying soil conditions. For the strongest pair, a deflection of 3 in. corresponds to a resistance of 80 lb., while for the weakest pair the same deflection corresponds to a resistance of 10 lb.

Friction between the tubes (*M*, Text-fig. 1) and the guide rods is a small and fairly uniform quantity so long as the guide rods and bearings are clean. The only other part of the apparatus where friction must be eliminated as far as possible is at the bearing between the probe and the base-plate. It would be possible to eliminate friction more thoroughly by the use of ball bearings in the two guide tubes and at the base-plate, but the plain bearings at present used have proved entirely satisfactory.

In some of the hardest soils, and where large stones are encountered, the  $\frac{1}{2}$  in. diameter rod of the probe tends to bend. This could be overcome by the use of a slightly thicker rod, for with increasing diameter of the rod, the critical buckling load increases much more rapidly than the soil resistance. It is undesirable to increase the diameter of the probe of the present instrument beyond  $\frac{5}{16}$  in. for general work, since the maximum force which can be exerted upon it is less than the weight of the operator.

Field work can be carried out very quickly. On cultivation experi-

ments, it has been usual to perform tests in four places over each plot, these four curves being recorded on the same chart. The testing of each such plot generally takes about 2 min., and the mean of the four curves usually gives a good approximation to the average condition of the plots. The variation due to experimental error is higher on light gravelly soils than on clays, and where many stones occur, the method loses much of its usefulness. In such cases, the standard error of one individual test may be up to 50 per cent. of the mean. The standard error for plots is reasonably low. In the gyrotilling experiment described in section V, standard errors for plots have been 8.7 and 9.4 per cent. of the mean of twelve plots. In some work on rolling, much lower errors have been obtained.

#### *Effect of speed of penetration on soil resistance*

The effect of speed of penetration on resistance is not a simple one. Experiments have been carried out on pure dry sand and on wet and dry soils, and the results obtained are variable. In only one case has a statistically significant effect been found. This was obtained in a careful test on a light prepared soil, uniformly compacted and with all the stones removed. The moisture content averaged 12.5 per cent. The speeds compared were  $\frac{1}{8}$ ,  $\frac{1}{4}$ ,  $\frac{1}{2}$  and 1 in. per sec. The average resistance at a speed of 1 in. per sec. (32.6 lb.) was significantly higher than the resistances at  $\frac{1}{8}$  in. per sec. (30.6 lb.) and at  $\frac{1}{4}$  in. per sec. (30.7 lb.) ( $P=0.05$ ).

The apparatus has been designed with a gearing which makes it possible to maintain a constant low speed of penetration and is unsuitable for tests on the effects of varying speeds, owing to the very small range over which speeds can be varied. By analogy with the effects of speed on the drawbar resistance of ploughs, it is probable that large increases in speed of penetration would give appreciable increases in the resistance to penetration. The design of the instrument eliminates this complication.

#### V. OUTLINE OF STUDIES IN A SOIL CULTIVATION EXPERIMENT

In 1933, a comprehensive series of experiments on deep cultivation by means of the Fowler "Gyrotiller" was begun on the University Farm. No attempt is made here to give any detailed account of the effects of gyrotilling on either the soil or the crop. The results of gyrotilling will be dealt with in collaboration with Mr F. H. Garner and Dr H. G. Sanders, who are responsible for the experiments, at some future date,

when experience has been gained during several years with different soils and crops. The object here is to illustrate the application of the apparatus previously described to studies of soil structure in connection with cultivation experiments, and in order to facilitate this, reference is made to studies on one particular experiment on Girton Allotment, a heavy clay field.

The experiment was laid out on a large scale in six randomised blocks, the gyrotiller cultivating the whole length of the field in strips approximately 42 ft. wide. (The working width of the machine is about 10 ft. 6 in.) The previous crop was seeds hay, after oats. The whole field was tractor ploughed to a depth of about 4 in. after removal of the hay crop, and gyrotilling was carried out on July 17, 1934, a few days after ploughing. The control plots were cultivated by horses at about the same time. The crop in 1933-4 was Wilhelmina wheat.

At the outset, it was clear that there were large differences of structure between the gyrotilled and control plots, and attempts were made to demonstrate and record these differences. The methods used have included percolation tests, soil fixation studies (Nicholson (7)), and sieving tests by a method similar to that described by Keen (1). All these methods have yielded useful data. The most striking difference between the gyrotilled and control plots was, however, the "looseness" of the former, and it seemed desirable to obtain some reliable measure of this property in order that the time of its disappearance might be noted. During the first year of the experiment, when tests aimed at the production of an instrument similar to that already described were in progress, experiments were made with a rifle and with a Service revolver.

It was found that the bullets from a 0.45 Webley revolver remained stable when fired into the clay soil, and, generally, a clean hole was left. The depth of penetration could easily be measured by pushing a piece of stiff wire down the hole and feeling the bullet at the bottom. The ammunition was a standard kind, and with this a 10 per cent. variation of muzzle velocity is not uncommon. In spite of this, and of certain

Table I. *Table showing results of penetration tests with revolver*

Date	Mean penetration cm.		Gyrotilled control	S.E. as % of general mean	Statistical significance
	Gyrotilled	Control			
29. i. 34	34.44	28.26	121.9	7.02	$P < 0.01$
16. iv. 34	29.45	24.66	119.4	8.05	$P < 0.05$
30. vii. 34	24.04	23.38	106.7	11.74	Insignificant
25. x. 34	24.33	24.48	99.4	6.45	Insignificant
22. i. 35	30.37	29.35	103.5	4.02	Insignificant

other disadvantages, the method has yielded results which are both useful and interesting. The results are presented in Table I and are illustrated in Text-fig. 3.

The difference of penetrability as measured by the revolver had disappeared so far as to become statistically insignificant by the end of July 1934, *i.e.* approximately a year after the operation of gyrotilling. But at this date, wide differences in soil structure were still present and

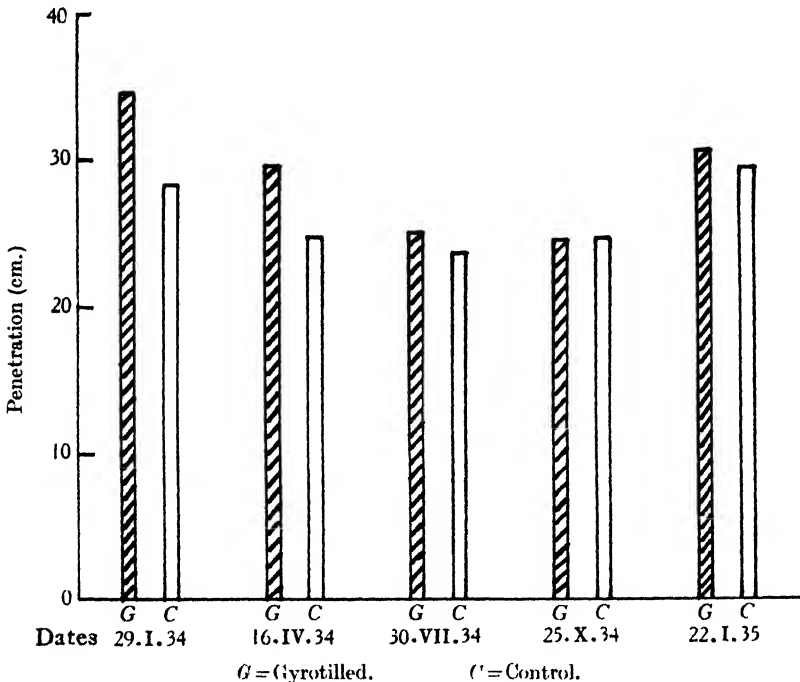


Fig. 3. Graph showing penetrability of revolver bullets on gyrotilled and control plots at various dates, after gyrotilling on July 17th, 1933.

demonstrable by other methods. An apparatus, similar in principle to that described earlier in this paper, but differing in some details of construction, was used to measure the resistance of the soil on the plots at various depths, two days after the revolver tests of July 30. The depth-resistance curves obtained are plotted in Text-fig. 4. Over the whole range of depths, the difference between gyrotilled and non-gyrotilled plots was highly significant. The maximum difference between the gyrotilled and control plots was reached at about the 5th inch. It then declined rapidly down to 18 in.

The difference in the nature of the results obtained by the revolver



and by the slow-moving probe was to be expected from theoretical considerations. The penetrability of the revolver bullets is roughly inversely proportional to the soil density, where other conditions such as moisture content are constant. This has been confirmed by experiments on large boxes of prepared soil. The penetrability of the revolver bullets thus gives a measure of Compactness. So long as the soil on the gyrotilled plots remained so loose as to be appreciably less dense than that on the controls, the revolver gave a measure of this effect. After a year, the

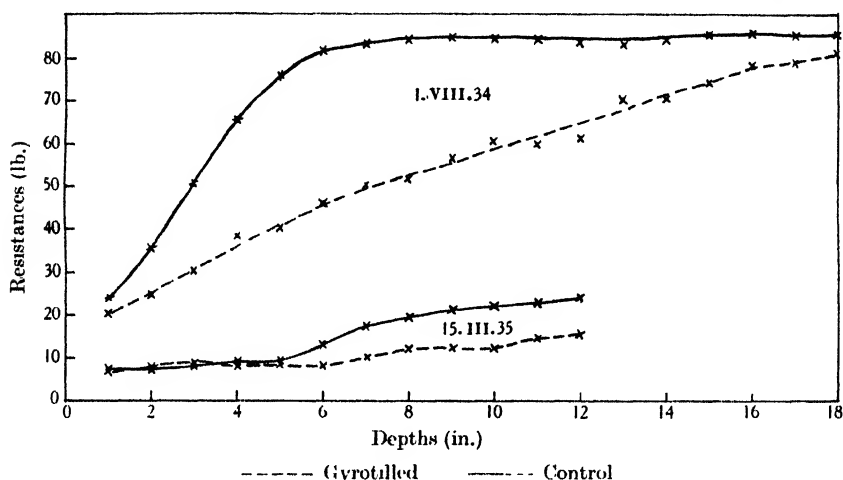


Fig. 4. Graph showing mean depth-resistance curves at two dates after gyrotilling in July 1933.

soil had settled down considerably and there was no measurable difference in the compactness of the soil on the two series of plots, but subsequent tests with the apparatus for the measurement of soil resistance have shown that the differences in resistance persist much longer. This is easily understood. Although the soil crumbs and clods settle down, planes of weakness remain and are easily demonstrable.

On October 24 and 25, 1934, the plots were ploughed in preparation for spring oats. A 10/20 H.P. International tractor and two-furrow Ransomes' "R.S.L.D." plough, fitted with "Y.L." breasts and shares were used. The drawbar pulls on the plots were measured by means of a Watson drawbar dynamometer.<sup>1</sup> The use of this dynamometer is fully described by Keen and Haines(8) in a previous volume of this *Journal*.

<sup>1</sup> This dynamometer was lent by the Institute for Research in Agricultural Engineering, Oxford University, and the kindness of the Director, Dr Denham, and his colleagues in adjusting the dynamometer and advising upon its use is gratefully acknowledged.

The dynamometer was calibrated in a 5-ton testing machine at the University Engineering Laboratory. It was found to be completely satisfactory over a range of pulls from 1200 to 3600 lb., and the experimental pulls fell well within this range. There was a highly significant difference between the drawbar pulls on the gyrotilled and control plots (Table II). It was also found that with the same setting of the plough, the average depth of work was greater on the gyrotilled plots.

Table II. *Summary of dynamometer tests*

Test	Control plots	Gyrotilled plots	Significance of differences
Mean drawbar pulls (lb.)	1729.8	1556.4	$P < 0.01$
Mean depths of ploughing (in.)	5.66	6.44	$P < 0.01$
Mean pull per square inch of cross-section of furrow slice (lb.)	16.98	13.43	$P < 0.01$
Horse-power developed	10.6	9.4	$P < 0.01$

The connection between implement drawbar resistance and the resistance to the penetration of the pointer of the apparatus previously described is demonstrated by a significant correlation between the drawbar pulls recorded in ploughing and the mean resistances over the first 6 in. as recorded by the tests with the apparatus described. Table III shows the correlation coefficients between the October 1934 drawbar pulls and the probe resistance tests of August 1934 and of March 1935.

Table III. *Significance of correlation coefficients*

	Degrees of freedom	Correlation coefficients	
		Oct. 1934 drawbar pulls with Aug. 1934 probe resistance	Oct. 1934 drawbar pulls with Mar. 1935 probe resistance
Blocks	5	+ 0.700	+ 0.794
Gyrotilled v. Control	1	+ 1.000	+ 1.000
Error	5	+ 0.047	+ 0.050
Total	11	+ 0.728*	+ 0.710*

\* Significant  $P < 0.01$ .

The significant positive coefficients for the total correlation indicate that there is a general correlation between the resistances obtained by the two methods. The coefficients for blocks just miss the required level of significance. The value of the correlation coefficient required for significance is high on account of the low value of  $n$  ( $n=4$ ) which must be used to determine it. The error correlation is very low. This indicates that when the effects of blocks and of the gyrotilling treatment have been accounted for, there is no correlation between individual plot

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readings obtained by the two methods. This is to be expected. Each plot covers an area of approximately 1 acre. The dynamometer tests covered approximately one-tenth of this area, and the tests with the probe covered only a minute fraction of the ground. It is more than likely that in many cases, none of the tests with the probe was on the strips tested with the dynamometer. It would thus be surprising if a high error correlation were obtained.

Eden and Maskell<sup>(10)</sup> have demonstrated a connection between soil resistance and plant growth. This connection has been confirmed in the experiments on gyrotilling and also in other cultivation experiments. Table IV shows the correlation coefficients between a germination count on the spring oats in April 1935, and (a) the resistances obtained in the October 1934 drawbar pulls and (b) the March 1935 probe tests.

Table IV. *Significance of correlation coefficients*

	Degrees of freedom	Correlation coefficients	
		Apr. 1935 germination count with Oct. 1934 drawbar pulls	Apr. 1935 germination count with Mar. 1935 probe resistance
Blocks	5	-0.87†	-0.77
Gyrotilling v. Control	1	-1.00	-1.00
Error	5	+0.36	+0.65
Total	11	-0.80*	-0.61†

\* Significant  $P < 0.01$ .

† Significant  $P < 0.05$ .

There was thus a general negative correlation between the number of plants and soil resistance, but as in the case where the two methods of measuring soil resistance were compared, the coefficient for error indicates that there is no such correlation between the readings for the individual plots.

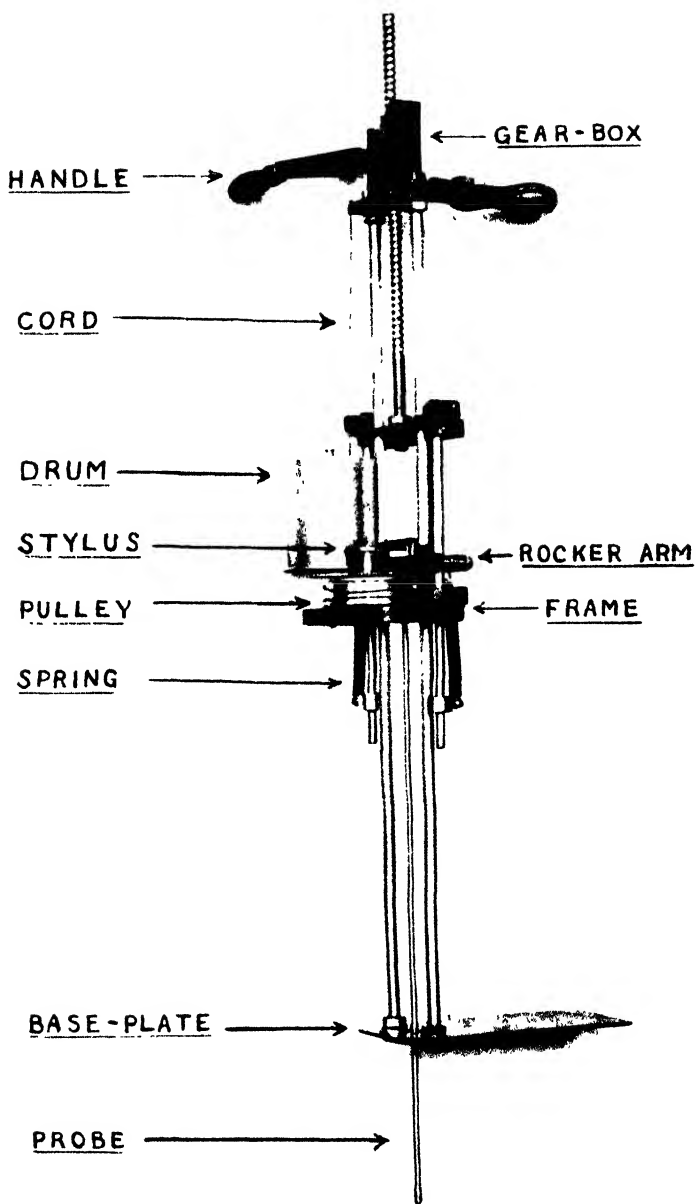
## VI. SUMMARY

The need for physical measurements of soil structure to assist in studies of the relation between cultivation implements and plant growth is pressing.

The nature and measurement of soil compactness and consolidation are briefly discussed. Measurements of soil resistance give an indication of consolidation rather than of compactness.

An instrument used for the measurement of soil resistance is described, with some notes upon its performance.

A brief outline is given of studies on soil structure in relation to



Soil resistance recorder.



gyrotilling. It is shown that the soil-resistance apparatus described gives results similar to those obtained by the use of the drawbar dynamometer in ploughing. A relation between soil resistance and plant growth is demonstrated.

I wish to acknowledge my indebtedness to Prof. F. L. Engledow, C.M.G., M.A., for constant guidance in carrying out this work. My thanks are also due to Prof. G. I. Taylor, F.R.S., and Dr H. G. Sanders, M.A., for helpful advice.

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# THE INFLUENCE OF ENVIRONMENTAL TEMPERATURE ON THE RESPIRATORY RHYTHM OF DAIRY CATTLE IN THE TROPICS

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(With One Text-figure)

THAT extreme environmental temperatures have a marked influence on metabolism is a well-established fact. The early investigators, Kellner, Atwater, Armsby and others, studied the energy income and output of farm animals in relation to various conditions of the environment, and from these studies the principles of basal metabolism, critical temperature, and physical and chemical regulation of body temperature have evolved.

From the point of view of live-stock breeders in the Tropics there are certain interrelationships of the above that are of considerable importance to them as husbandrymen. A brief review of these relationships follows:

(1) As the external temperature rises above the critical temperature the animal organism facilitates the elimination of body heat by physical regulation in an endeavour to maintain normal body temperature.

Physical regulation is effected by radiation and conduction of heat from the skin and elimination of heat as latent heat of water vapour from the skin and lungs. All three function simultaneously at all temperatures; however, as the external temperature rises, a greater part of the temperature control passes, in ruminants, from radiation and conduction to emission of heat as water vapour from the lungs. There is, however, a definite range of temperature above the critical through which physical regulation can maintain normal body temperature. As the external temperature rises above the upper limit of physical regulation, heat is retained in the body, and body temperature rises as a result. This in turn increases cellular activity with a corresponding increase in heat production and loss of energy.

(2) With increasing high air temperatures the burden of physical regulation in ruminants is forced upon the lungs, which, accelerating their rhythm, eliminate increased quantities of the end-products of

katabolism, heat, carbon dioxide and water. In relating lung activity to metabolism Armsby(1) states: "It is a familiar observation that the rate of ventilation of the lungs varies with varying activity of the body cells."

(3) Apart from increased metabolism at high temperatures there is an increase in heat production with rising temperature due to the additional energy expended in various processes of physical regulation. The data presented in Table I give an idea of the increased muscular activity with rising temperature.

(4) The thermal environment may also increase metabolic rate of animals through the condition of the atmosphere as regards moisture and sunlight. Rubner(5) has shown how high moisture content of the air, relative humidity 60 per cent., may increase considerably the metabolism in dogs and other animals by lowering the rate of evaporation of the water given off from the lungs and skin. In this way high humidity lowers the upper limit of physical regulation.

(5) Direct sunlight may impart a considerable amount of heat to the body, which in addition to high air temperature and humidity in the Tropics causes considerable discomfort and loss of energy if shade is not provided.

(6) That long and dense hair coat is detrimental to physical regulation at high temperatures is evident, as it hinders both radiation and conduction of heat from the skin. Forbes *et al.*(3) have clearly demonstrated the effect of long hair (normal winter coat) and short hair (clipped winter coat) on physical regulation. The shorn steer in the Forbes experiment was less affected by high temperatures as it could more readily throw off excess heat, *i.e.* clipping raised the upper limit of physical regulation.

The above factors interest the breeders in the Tropics in the manner in which they influence the metabolic level irrespective of the influence of work and digestion.

That these factors influence metabolism in accordance with the position of the critical temperature and range of physical regulation are established facts. The lower the critical temperature and upper limit of physical regulation the more readily will the metabolic rate be accelerated by tropical temperatures, and conversely, the higher the critical temperature and upper limit of physical regulation the more resistance will the animal have to tropical atmospheric conditions.

Although the critical temperature and range of physical regulation are influenced by feeding and extreme external conditions they are both



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determined by inheritance, varying with the species and showing a reasonable degree of variation above and below the mean in individuals. They form a part of the constitution of cattle and determine in a large degree the adaptability to specified thermal environments.

Armsby(2) places the critical temperature of ruminants at approximately 56° F. (approx. 13° C.), citing the "work of digestion" as responsible for a large amount of heat necessary to maintain body temperature. Hays(4), studying the influence of atmospheric temperature on butterfat percentage, gives 70° F. (21° C.) as the temperature above which the increased metabolism was sufficient to influence mammary secretion.

It is the object of the present study to gauge the influence of external temperature and humidity on European and Zebu dairy cattle in an endeavour to explain, in part, the poor results characteristic of the former in the Tropics.

#### MATERIAL

In organising the material for this study the dairy herd owned by the College was used. This herd of seventy-three head is in a large part made up of Holstein Zebu and Guernsey Zebu cross-breds of varying degrees of European blood. It also contains twenty head of pure-bred Holstein Freisians, including eight imported animals. The College herd also contains several Zebu of the Guzarat type.

For this study only the cows that were in milk during January 1935 were used as source of data. At this time there were five pure-bred Holsteins, all imported, eleven Holstein Zebu cross-breds, three-quarter to fifteen-sixteenths Holstein, and two Zebu.

The herd is subject to the outdoor regime the year round. The cattle are on pasture both night and day, entering the stable in the early morning and evening for milking and to receive their grain ration. The pastures are hilly and amply supplied with natural shade. Viçosa is at 646 m. altitude and 20° south latitude. The fact that the school has been placed at a high altitude as a desirable health environment for the students makes it also a better than average thermal environment for European cattle, a point that should be remembered in making comparisons.

#### METHODS

Noticing the accelerated respiration of all the cattle during the hot summer months and especially the exhausted appearance of the pure-bred European stock, the writer took respiration counts on various

individuals at varying degrees of air temperature in order to determine the relation of the one with the other.

Respiratory counts were taken twice daily, at 7 a.m. and from 2 to 3 p.m. At 7 a.m. the cattle had been in the stable about an hour and had had time to recuperate from walking in from the night pasture, which was, however, close by. Between 2 and 3 p.m. the counts were taken in the pasture. This is the hottest hour of the day, and respiratory counts were usually easy to obtain as the cattle were usually together under shade. Counts were taken only after the animals had been quiet and under shade for some time previous. The respiratory rhythm was obtained from flank movements. These could be observed from a distance without disturbing the animals. Observations were made over half-minute periods and twice verified. A stopwatch was used in determining time intervals. At the higher temperatures the respiration efforts were often so strong that the whole body was forced into rhythm with the flank movements.

Repeated observations were made on other individuals of the school herd, and visits were made to neighbouring herds for observations on Zebu cattle. These extra observations confirmed in a general way the data here presented but were not included in the study as they were not of the same age group or under the same management.

Counts were also taken in May on the same individuals although some were not then in milk. May is a month of comfortable air conditions; it is the time of the year that European cattle "pick up".

From the meteorological records obtained at the school, the number of hours per month at which the air temperature was at or above the point of increased metabolism,  $21^{\circ}\text{C}$ ., was calculated and recorded for the years 1931-4 inclusive. For the same period the mean relative humidity was determined for the hours of the day that the temperature was at or above  $21^{\circ}$ .

## RESULTS

Table I gives the respiration counts for the various groups:

In the pure-bred group of Table I it will be noted that the cows Creamy and Laurel have rapid rhythm at comfortable temperatures which raise the average for this group above what may be considered normal for the Holstein Freisian breed. At high temperatures,  $29$  and  $36^{\circ}\text{C}$ ., they apparently do not unfavourably influence the group average. Their breathing, especially Creamy's, lacked depth.

In the group of Holstein cross-breds the average is not unfavourably

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influenced by any abnormal respirations. Brasileira, however, is remarkable for her slow and deep breathing. In this group there are three cases in which respirations decreased in going from 11 to 19°. The same occurred with Zeita of the Zebu group. As all counts were verified the writer has no explanation for these facts.

Table I. *Respirations per minute for pure-bred Holstein, Zebu and cross-bred cattle at varying degrees of external temperature*

	May		January				May		January		
	a.m.	p.m.	a.m.	p.m.	p.m.		a.m.	p.m.	a.m.	p.m.	p.m.
	11°	19°	23°	29°	36°		11°	19°	23°	29°	36°
Pure-breds:						Cross-breds:					
Pearl	21	25	28	92	118*	Brazileira	14	18	24	64	92
Laurel	30	30	56	104	116	Diamantina	18	20	30	92	98
Creamy	44	42	62	98	108	Dora	20	32	28	70	86
Oeltje	24	28	42	94	92	Gaucha	20	21	38	76	98
Margaret	21	26	34	74	102	Nisa	22	30	39	78	—
Zebu:						Luccerna	23	22	31	82	—
Zelandia	21	24	26	34	46	Espera	23	18	24	70	86
Zeita	25	22	28	35	—	Genica	20	18	24	60	76

\* In an adjoining pasture two yearling pure-bred bull calves had respirations of 124 and 126 respectively. These were the highest counts registered.

Furthermore, it should be noted that at the comfortable temperatures 11, 19 and 23°, there is a general tendency for the respiratory rates to remain in more or less the same relative position in which they stand at the lowest temperature. At higher temperatures this relationship does not always maintain itself. An important observation is that some of the cross-breds had counts as low as the Zebu stock at comfortable temperatures, but upon reaching tropical temperatures the cross-breds without exception deviated from the Zebu curve.

The averages for the groups are given in Table II and plotted on graph as shown in Fig. 1.

Table II. *Average respirations per minute at varying degrees of external temperature*

	11°	19°	23°	29°	36°
Pure-bred Holstein*	28.0	30.2	44.4	92.4	107.0
Cross-bred Holstein	20.0	22.4	29.8	74.0	89.3
Zebu	23.0	23.0	27.0	34.5	46.0

\* Average weight: pure-breds 565 kg., cross-breds 436 kg., Zebu 432 kg.

From Table II and Fig. 1 it is evident that the normal rate of respiration is approximately the same for all groups, the rate at 11° being

considered normal. Eliminating Creamy and Laurel from the pure-bred group the averages would be 22.0, 26.3 and 34.6 respirations at 11, 19 and 23° respectively.

There is a slight increase in rhythm in going from 11 to 19°, but the first appreciable increase occurs in the pure-bred group between 19 and 23°. The increases for all groups in the 12° range, 11-23°, may be attributed to normal physical regulation in which the energy loss is

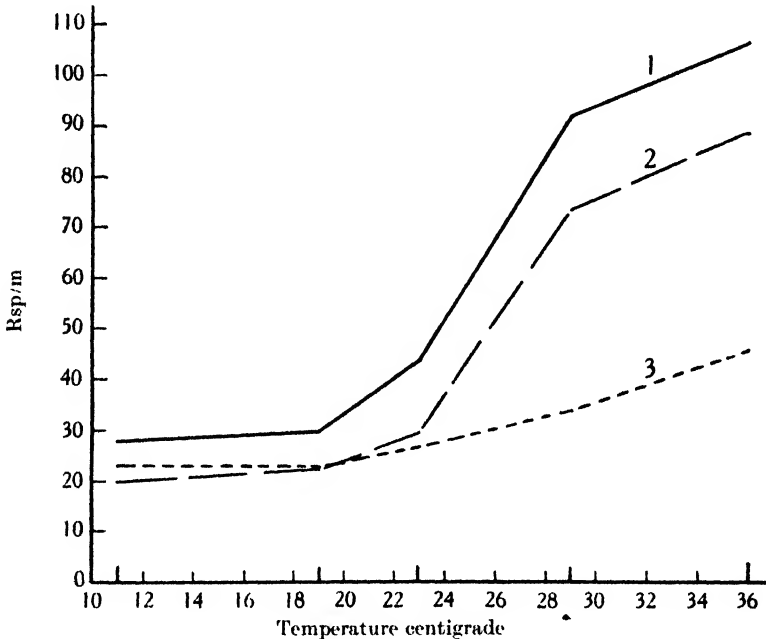


Fig. 1. Respirations per minute at varying degrees of external temperature.

1. Pure-bred Holstem. 2. Crossbred Holstein Zebu. 3. Zebu.

non-detrimental to normal functions of production or growth. Exception to this may be taken with the pure-bred Holstein cattle where the increase was greatest.

On the other hand the pronounced increase in rhythm for the pure-bred and cross-bred groups between 23 and 29°, a range of only 6°, cannot but indicate an increased metabolic rate accompanied with a considerable loss of energy through greatly increased muscular activity and possibly through increased cellular activity as a result of heat retention in the body.

Over the same range the increase in respiratory rate for the Zebu

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cattle was slight, and although this may represent a loss of energy it appears to be well within the range of physical regulation for this species.

From 29 to 36°, a range of 7°, the rhythm again increases, but not as much as in the previous range. The important observation in this range is that at 36° the Holstein and cross-bred groups are approximating their maximum muscular efforts of physical regulation through the lungs. This, however, is not indicated in the Zebu curve.

That the energy expended in physical regulation and increased metabolism at the higher external temperatures is energy that at comfortable air temperatures would be used for other purposes as work, growth, fattening or milk production follows the energy balance concept. That is, as the original source of energy for any of the above functions is the net energy of foods, any factor that would tend to diminish the amount of net energy would thereby restrict any one or all functions to the extent that the energy available was diminished.

During the hot summer months the pure-bred cattle would often seek the shade by 9 a.m. and remain there till 3 p.m., when brought in for milking. The writer has also observed that while under shade, with high atmospheric temperatures, the cattle do not lie down but stand, as this position facilitates rapid respiration. In this respect the breeder in the Tropics should realise that his pure-bred or grade European cattle while under shade during the heat of the day are not resting but may be expending more energy than his Indian cattle calmly pasturing. When the temperature approximates blood heat, accompanied with high humidity, the cattle become quite exhausted, just living.

The difference in the manner in which European and Zebu cattle respond to high air temperatures may on first analysis be attributed to their most striking contrasting character—the long hair coat of the European cattle and the short coat of Indian cattle. That this is an important factor has been shown by Forbes *et al.* (3). That there are also other factors responsible is indicated in a study of the data in the cross-bred group. The cows Brasileira, Diamantina and Dora have short Zebu-type coats, while Genica and especially Espera have more than average amount of hair. Their respiratory counts, however, are not in relation to the length of hair coat. The respiration of Genica is the lowest of the cross-bred group, and that of Diamantina one of the highest.

The degree to which the loss of energy as the result of high environmental temperatures is detrimental to milk production, growth, fattening or work is in direct relation to the time period the cattle are subjected

to tropical conditions. Short periods of extreme thermal conditions may be supported by most warm-blooded animals without endangering health or body functions. It is common observation, however, that prolonged periods of the same abnormal conditions may seriously affect health and production. The animal organism tends to adjust itself to the changed conditions to the extent it is capable of. That in the Tropics certain breeds are able to do this better than others is used as a basis for selection of breeds.

In order to determine the time period, use was made of the meteorological records obtained at the school. Using the same records it was possible to determine, with a fair degree of accuracy, the relative humidity during the period of day when the air temperature was at or above 21°. The results by months over the 4-year period 1931-4 inclusive are given in Table III.

Table III. *Hours of tropical temperature and mean relative humidity, Viçosa, Minas Gerais, Brazil*

	1931		1932		1933		1934		4-year average	
	Hours	Rel. hum. %	Hours	Rel. hum. %	Hours	Rel. hum. %	Hours	Rel. hum. %	Hours	Rel. hum. %
January	434	80	310	80	372	84	387	80	378	81
February	294	81	336	80	322	82	357	75	327	79
March	341	82	325	79	294	72	325	76	321	77
April	270	78	225	72	150	73	240	76	221	75
May	135	69	105	66	75	62	105	70	105	67
June	60	61	45	67	30	52	82	59	54	59
July	70	57	46	60	23	62	39	50	44	57
August	46	52	101	57	93	55	124	63	91	57
September	120	63	195	62	150	61	150	68	154	63
October	248	71	248	72	201	69	163	67	215	70
November	270	74	315	75	225	70	308	76	279	74
December	372	77	341	83	310	83	387	83	352	81
Totals	2660		2592		2245		2667		2541	

From Table III it is seen that some time during the day even in the cool months the temperature reaches summer heat. It is during the months of May to August that European cattle "do well" in Viçosa. During the remainder of the year they suffer from high temperatures and humidity.

Summing the monthly figures the yearly total of hours that the cattle are subjected to tropical heat is found to be considerable even for Viçosa, a mountain village. Over the 4-year period this represents 254.1 10-hour days per year that European dairy cattle are losing energy at the expense of production, weight or growth. For the larger part of Brazil and other

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regions of the tropical world, where an endeavour is being made to breed European cattle as pure-breds or high grades, the total tropical hours per year must be considerably higher than the figure for Viçosa, and the cattle suffer a correspondingly greater loss of energy.

### CONCLUSIONS

This paper represents a study of the influence of tropical environmental temperatures upon metabolism, as indicated by rhythm of respiration, of dairy cattle in Minas, Brazil. The following conclusions have been reached:

High external temperatures and humidity influence the metabolic level of European and Indian cattle in unlike ways, indicating a species difference in manner of response to tropical temperatures.

Between 23 and 29° C. the metabolic rate of pure-bred and high-grade cross-bred European cattle is considerably increased, while that of Indian cattle is only slightly increased.

Between 29 and 36° the metabolic rate is again increased. At 36° the European cattle have apparently reached their maximum efforts of physical regulation through the lungs. This, however, is not indicated in the respiration curve for Zebu cattle.

The respiration curve for the cross-breds, although lower than that with the pure-bred group, is of the same type as the latter.

In Viçosa, Minas, cattle are exposed to an average of 254.1 10-hour days per year of atmospheric temperatures and humidity that are detrimental to milk production and growth of European cattle.

The author suggests that the loss of energy in dairy cattle as the result of high temperatures is in a large way responsible for the low production records of European dairy cattle in the Tropics.

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# STUDIES ON THE RELATION BETWEEN CULTIVATION IMPLEMENTS, SOIL STRUCTURE AND THE CROP

## II. THE EFFECTS OF THE FOWLER "GYROTILLER" ON THE SOIL

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(With Plates II and III and One Text-figure)

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### I. INTRODUCTION

It is not ploughing, it is not digging, it is not harrowing, raking, hoeing, rolling, scarifying, clod-crushing, scuffling, grubbing, ridging, casting, gathering that we want: all these are the time-honoured, time-bothered means to a certain RESULT. That result is—a seed bed. HOSKYNs (1845).

THE possibilities of rotary cultivation as a method of preparing seed beds have engaged the attention of agriculturists ever since mechanical power became available to the farmer. The idea of rotary cultivation is an attractive one, but, until recent years, attempts at producing a rotary cultivator which could advantageously be used by the ordinary arable farmer had met with partial or complete failure. The internal combustion engine has many advantages over the steam engine as a source of power for rotary cultivation, and many attempts have been made to adapt it to this purpose. It has been successfully used in small rotary cultivators, of which the common type is the Simar "Rototiller" (1). These small rotary cultivators now occupy an important position in the equipment of many horticulturists and market gardeners; but such machines are not suitable for use on an ordinary arable farm.



During recent years, two types of rotary cultivator which may well assume a permanent place in farming practice have been evolved. The first type is a tractor attachment in which the tillers, like those of nearly all the rotary cultivators which have been invented during the past 80 years, work about a horizontal axis in the manner envisaged by Hoskyns. The second type, the Fowler "Gyrotiller", works on an entirely new principle. The tillers work about an axis which is nearly vertical. Pl. II gives a general view of the machine. This cultivator has become available to farmers in most of the more important arable areas where steam cable tackle sets have been used in the past. Contractors own the machines and do the work at a price of thirty shillings per acre, inclusive. Many farmers prefer the gyrotiller to steam cable tackle because they are saved the bother and expense of supplying and carting coal and water, and also because the gyrotillers cultivate the headlands and corners.

It has not been proved that rotary cultivation is the most "efficient" method of applying power to cultivation operations. It is true that in the case of a tractor pulling a plough, a high proportion of the power is wasted in its transmission from the engine to the implement drawbar. In spite of this, it is doubtful whether, except under special circumstances, a seed bed can at present be produced with the expenditure of less work by rotary cultivation than by the traditional methods. This will not be proved until it can be shown that the power which is delivered to the tillage bodies of rotary cultivators is applied to the work of cultivation by those bodies as efficiently as is the power which is delivered to plough bodies. But apart from any question of mechanical efficiency, the rotary cultivator may have a permanent place in arable farming because it is able to carry out certain operations more effectively than any other implement.

Keen (2) has stressed the importance of studies to determine whether the work done by rotary cultivators differs essentially from that done by the traditional methods. Studies must be made to determine in what circumstances gyrotilling may be done with advantage. It is certain that rotary cultivation must be carried out with as much discrimination and care as any other cultivation. A few examples of unsatisfactory work on heavy soils which were too wet have already been experienced.

In 1933, a 170 H.P. Fowler Gyrotiller was used on the Cambridge University Farm for breaking up the bastard fallows. Steam cable tackle had hitherto been used for this work. The gyrotiller was found satisfactory, and has been used in 1934 and 1935 in preference to steam tackle. During these three seasons, four experiments aimed at securing

information concerning the effects of the gyrotiller on the soil and the crop have been laid out. Two of the experiments are on light land and two on heavy. An account of these experiments is given below, but this paper is essentially concerned only with the effects of gyrotilling on the soil. The experiments will not be complete until these effects are no longer perceptible. But the gyrotiller has been widely employed by farmers, and frequent enquiries as to its effects are received. It has therefore seemed desirable to publish the experimental evidence so far obtained. The effects produced on the crops are referred to by Dr H. G. Sanders (3) elsewhere.

## II. THE EXPERIMENTS

Table I gives an outline of the experimental work which has been done. Part of this outline has already been filled in by section V of an earlier paper (4). In that paper, an account has been given of the following tests on one of the fields, Girton Allotment:

(1) Tests of soil compactness with a revolver. A description is given of the technique, and the results of five tests at various dates after gyrotilling are included.

(2) Tests with a soil resistance recorder. A description is given of the construction and operation of the recorder, with the results of a test carried out 12 months after gyrotilling.

(3) Tests with a drawbar dynamometer when the field was tractor ploughed 15 months after gyrotilling. A summary of the results is presented.

These tests are therefore only briefly referred to in the present paper.

### *Notes on the work done by the gyrotiller*

#### (1) *Girton Allotment*

This field is typical of a large area of gault clay land in west Cambridgeshire. Such land generally benefits from being burst up by cultivators when it is very dry, and it is good practice to use steam cultivators on the fallows or bastard fallows about once every 4 years. The gyrotiller worked when the soil was dry. It brought a few large clods of subsoil to the surface. At the time of drilling the wheat, 3 months after gyrotilling, the gyrotilled plots were very "open". in spite of the large number of cultivations which had been performed. The weather had been exceptionally dry, and the seed bed on the gyrotilled plots was looser than the farmer generally likes it for wheat. Very little rain fell during the first 12 months after gyrotilling, and this fact may to

Table I. *Table giving an outline of the experimental work on four fields on the University Farm*

Soil	Heavy Gault Clay		Light Gravelly Loam	
	Girton Allotment	Longfallen	Bunkers'	Dry Field
Date gyrotillled	July 1933	August 1934	August 1934	August 1935
Lay-out	6 randomised blocks	8 randomised blocks	8 randomised blocks	7 randomised blocks
Treatments	(1) Gyrotillled (2) Horse cultivated	(1) Gyrotillled (2) Horse ploughed	(1) Gyrotillled (2) Horse ploughed and subsoiled	(1) Gyrotillled (2) Horse ploughed (3) Horse ploughed and subsoiled
Size of single plot	40 ft. × 200 yards	40 ft. × 90 ft.	40 ft. × 90 ft.	30 ft. × 90 ft.
Previous crop	Oat and tare hay after oats	Wheat after wheat	Wheat after potatoes	Wheat after potatoes
Chief operations and observations after gyro-tilling	1933: Broadshared Aug. Drag harrowed Sept. Horse cultivated and harrowed; wheat drilled Nov. Permeability test 1934: Revolver test (1st) Jan. Spring cultivations; 2nd revolver test Apr. Wheat harvested; 3rd revolver test July 1st test with resistance recorder Aug. Ploughed with tractor; drawbar pulls recorded by dynamometer; 4th revolver test 1935: 5th revolver test Jan. 2nd test with resistance recorder; horse cultivated 3 times; sieving tests; spring oats drilled May Undersown with red clover; 3rd resistance test Aug. Oats harvested Sept. 4th resistance test	1934: Tractor cultivated twice Oct. Tractor cultivated; 1st revolver test; 1st resistance test; beans drilled 1935: Jan. 2nd revolver test Aug. Beans harvested Sept. Tractor ploughed Oct. 3rd revolver test; 2nd resistance test	1934: Sept. Drag harrowed Oct. 1st resistance test Nov. Farmyard manure ploughed in 1935: Mar. Tractor cultivated twice Apr. Drag harrowed; Cambridge rolled; sugar-beet drilled May 2nd resistance test Sept. 3rd resistance test	1935: Sept. Resistance test

some extent account for the remarkable persistency of the looseness of the soil on the gyrotilled plots on this field.

(2) *Longfallen*

The soil of this field differs little from that of Girton Allotment. The initial effect of gyrotilling on the two fields was similar.

(3) *Bunkers' Field*

The soil of this field is a poor gravel and has a marked tendency to form a hard pan just below the normal depth of cultivation. The gyrotiller succeeded in breaking up this pan and worked the soil to a depth of 12–16 in. on most of the field. It left the soil “fluffy”, but this initial fluffiness was not as persistent as the looseness on the heavy land, and disappeared after a few cultivations.

(4) *Dry Field*

The soil of this field is very similar to that of Bunkers' Field. When the gyrotiller was at work in August, the ground was very hard and dry. In some parts of the field, the gyrotiller was unable to work the soil to a depth of more than 8–12 in. The ploughing and subsoiling were done about a month later, after a considerable quantity of rain had fallen. The land was then much softer, and the subsoiler succeeded in penetrating effectively in a way that would have been impossible at the time when the gyrotiller was at work.

### III. TESTS OF SOIL COMPACTNESS

(1) *Girton Allotment*

It has been shown in a previous paper<sup>(4)</sup> that the penetrability of revolver bullets may be taken as a rough measure of the “compactness” of the soil, where compactness is regarded simply as the density of the mass of soil. The tests with the revolver on Girton Allotment<sup>(4)</sup> showed that the gyrotilled plots were still less compact than the controls after 9 months, but no difference was measurable by the revolver after 12 months. During this time, the soil on the gyrotilled plots had gradually settled down until the large air spaces which at first existed were almost obliterated. Nicholson<sup>(5)</sup> has demonstrated differences in subsoil structure on the gyrotilled and control plots 15 months after gyrotilling by the use of a method whereby the soil is fixed in a mixture of paraffin wax and naphthalene. This method does not give an accurate picture of the soil *in situ* on account of the shrinkage entailed in a pre-

liminary drying process which must precede impregnation with the fixing mixture. But examination of the fixed sections has led Nicholson to conclude that small cavities still persisted in the soil *in situ* after 15 months. The revolver test is not sufficiently sensitive to be influenced by cavities when they become very small.

(2) *Longfallen*

Table II. *Table showing results of penetration tests with revolver on Longfallen at various dates after gyrotilling in August 1934*

Date	Mean penetration cm.		Gyrotilled Control %	S.E. as % of general mean	Significance of difference
	Gyrotilled	Control			
3. x. 34	25.4	22.2	114	3.5	$P < 0.01$
18. i. 35	29.8	25.9	115	5.2	$P < 0.01$
2. x. 35	27.3	27.3	100	4.5	—

*Discussion of Table II*

The results obtained on Longfallen confirm those obtained on Girton Allotment. In the early days of the experiment, the extreme looseness of the soil on the gyrotilled plots was easily demonstrable by the revolver tests, but as on Girton Allotment, the difference between gyrotilled and control plots no longer affected the revolver penetrabilities after 12 months. The last test (on 2. x. 35) was done on the subsoil, with the top soil removed, in order that any differences which might have existed in the subsoils should not be masked by the bullets penetrating the top soil, where no measurable differences could be expected.

## IV. TESTS OF SOIL RESISTANCE

The soil resistance tests have been carried out chiefly by use of the resistance recorder described in a previous paper (4). In addition, tests of ploughing draughts with a drawbar dynamometer have been carried out on Girton Allotment (4). All the results presented below have been obtained with the special resistance recorder. Throughout the work, the probe used has been a  $\frac{1}{4}$  in. diameter steel rod with a  $\frac{5}{16}$  in. diameter point (4). The readings for each individual plot have been obtained by taking the mean of four samples. The figures for the individual plots have been subject to statistical analysis, and the significance of the differences is expressed by the usual notation<sup>1</sup>. The results obtained with this apparatus on Girton Allotment are given in Table III.

<sup>1</sup> Fisher, R. A. *Statistical Methods for Research Workers* (1935). London: Oliver and Boyd.

Table III. *Girton Allotment. Table showing mean resistances (lb.) to penetration of probe at various dates after gyrotilling in July 1933*

	1. viii. 34 after 12 months		15. iii. 35 after 19 months		31. v. 35 after 21 months		24. ix. 35 after 25 months	
Depths in.	Con- trol	Gyro- tilled	Con- trol	Gyro- tilled	Con- trol	Gyro- tilled	Con- trol	Gyro- tilled
1	24.3	20.0	6.2	5.9	22.6	21.1	8.9	7.3
2	35.2	24.4	7.1	8.1	27.8	31.7	11.3	9.2
3	50.7	30.0	8.5	8.8	30.8	38.8	15.2	13.3
4	63.2	37.9	9.0	7.7	29.8	36.7	19.5	21.2
5	72.6	40.7	8.6	8.0	31.1	37.1	26.9	29.8
6	79.8	46.6	12.9	8.0	34.7	37.3	38.9	38.4
7	82.5	49.2	17.0	9.8	36.5	40.1	51.6	46.3
8	83.9	50.8	18.5	12.6	40.3	39.0	58.5	52.3
9	84.9	54.8	21.0	12.7	40.3	38.8	56.7	55.3
10	84.5	60.0	22.4	12.5	38.6	36.7	59.8	51.6
11	84.0	59.5	23.1	14.8	37.1	33.4	60.6	55.3
12	83.5	60.6	24.0	15.2	35.2	35.2	61.7	58.0
*Significance of gyro- tilled v. control com- parison	$G < 0$ ( $P < 0.01$ )		$G < 0$ ( $P < 0.05$ )		Insignificant		Insignificant	
Significance of inter- action between gyro- tilling and depths	Sig. ( $P < 0.01$ )		Sig. ( $P < 0.01$ )		Sig. ( $P < 0.01$ )		Insignificant	
Soil moisture content	Very dry		25.8 % 25.2 % (0.9 in.)		15.9 % 15.3 % (0.6 in.) 20.4 % 20.3 % (6-12 in.)		24.9 % 25.0 % (0.6 in.) 23.1 % 23.4 % (6-12 in.)	

### Discussion of Table III

When the tests were made on the wheat stubble 12 months after gyrotilling, it was found that there was a highly significant difference in resistance on the gyrotilled and control plots throughout the depth tested. It was somewhat surprising to find the difference even in the top few inches. This state must be attributed largely to an abnormally dry winter. The soil had never been thoroughly wet at any time during the preceding 12 months.

The drawbar tests which were made when the field was ploughed entirely confirmed the observations made above (4). There was a very noticeable difference in the furrow slices turned on the gyrotilled and control plots. On the gyrotilled plots, the furrow wall and bottom were broken and crumbled, while on the control plots they were clean-cut and shining (see Pl. III). It appeared that there might be a difference in the moisture content of the soil on the two series of plots, but the observations showed that there was no significant difference in moisture content.

The resistance tests made in March 1935, 19 months after gyrotilling, gave values very much lower than those obtained the previous August.

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This was partly due to the cultivations, but was certainly largely due to the higher moisture content of the soil. Moisture has a very important influence on the resistance to penetration of the probe of the apparatus. Generally speaking, the higher the moisture content, the lower the resistance, but the relation is not a simple one. The significance of the difference in the gyrotilled *v.* control comparison had fallen to  $P < 0.05$  for the mean of all depths from 0 to 12 in. This was due to the ploughing and cultivations which affected the top 6 in. of the soil. The interaction with depths was very significant. This was due to the fact that there was no difference between gyrotilled and control plots in the surface layers, but a very significant difference over the depths 6–12 in.

The third series of tests was made on May 31, when the soil was becoming dry. There was no significant difference between resistances on gyrotilled and control plots, but a significant interaction between these treatments and depths. The significant interaction is explained by the fact that the gyrotilled plots presented a slightly higher resistance at the surface and a lower resistance in the bottom layers. The gyrotilled plots had appeared drier than the controls throughout the spring, and it is thought that this may have caused the higher resistance at the surface. Determinations of the moisture content, however, showed

Table IV. *Longfallen Field. Table showing mean resistances (lb.) to penetration of probe at two dates after gyrotilling in August 1934*

Depths in.	10. x. 34 after 1½ months		1. x. 35 after 13 months	
	Control	Gyrotilled	Control	Gyrotilled
1	8.8	9.7	6.8	7.8
2	10.4	11.2	6.8	6.8
3	13.3	12.6	6.1	6.4
4	19.1	15.2	6.1	7.1
5	21.0	18.4	7.1	8.4
6	27.9	21.5	10.3	9.9
7	37.8	24.2	17.0	13.3
8	43.7	25.7	21.4	14.9
9	50.7	31.9	24.6	16.1
10	56.9	37.1	24.5	18.1
11	66.0	43.9	28.0	20.0
12	67.8	47.3	30.6	21.9
Significance of gyrotilled <i>v.</i> control comparison	$G < 0$ ( $P < 0.01$ )		$G < 0$ ( $P < 0.01$ )	
Significance of interaction between gyrotilling and depths	Sig. ( $P < 0.01$ )		Sig. ( $P < 0.01$ )	
Soil moisture content (0–12 in.)	19.4 %	19.1 %	23.0 %	23.4 %

differences which appeared too small to provide an adequate explanation in the present state of knowledge of the effects of moisture content on resistance.

The tests of September 1935 showed that the resistance in the lower layers of the gyrotilled plots was still slightly less than that on the controls, but the difference was quite insignificant.

#### *Discussion of Table IV*

The results obtained on Longfallen largely confirm those obtained on Girton Allotment. In the early days of the experiment there was a looseness of the soil down to 15 in. on the gyrotilled plots, and this looseness was easily demonstrable by both tests with the revolver and with the resistance recorder. A year after gyrotilling, when the revolver failed to show any significant difference, there was still a highly significant difference in the resistance of the subsoils to the penetration of the probe of the resistance recorder. This finding is in complete accord with the results obtained on Girton Allotment.

Table V. *Bunkers' Field. Table showing mean resistances (lb.) to penetration of probe at various dates after gyrotilling in August 1934*

	9. x. 34 after 2 months		31. v. 35 after 9 months		23. ix. 35 after 13 months	
Depths in.	Con- trol	Gyro- tilled	Con- trol	Gyro- tilled	Con- trol	Gyro- tilled
1	12.3	10.5	18.2	18.6	9.6	8.8
2	11.3	11.2	21.5	20.0	10.3	10.7
3	12.2	11.4	22.2	20.5	12.8	12.9
4	11.9	11.5	25.2	20.9	16.8	17.5
5	14.0	12.7	26.2	20.6	19.4	23.7
6	15.9	13.8	30.5	25.9	25.6	25.2
7	18.0	15.9	30.1	28.9	29.1	25.4
8	24.3	17.7	33.5	31.4	31.1	25.3
9	30.3	18.4	37.3	31.5	39.0	25.3
10	36.4	21.9	42.8	34.1	44.2	29.6
11	48.9	29.6	43.0	36.8	45.2	33.6
12	62.5	37.5	—	—	52.7	38.5
Significance of gyrotilled v. control comparison	$G < 0$ ( $P < 0.01$ )		0-12 in. Insig. 6-11 in. Sig. ( $P < 0.05$ )		0-12 in. Insig. 7-12 in. Sig. ( $P < 0.05$ )	
Significance of interaction between gyrotilling and depths	Sig. ( $P < 0.01$ )		Sig. ( $P < 0.01$ )		Sig. ( $P < 0.01$ )	

#### *Discussion of Table V*

On Bunkers' Field the high proportion of stones renders tests of soil resistance more difficult than on the heavy land. The revolver method for tests of compactness is quite impracticable, and the error of the tests with the resistance apparatus is high. The initial effect of gyrotilling on this land is similar to that on the heavy land, in that the soil is loosened throughout the cultivated layer. The general looseness does not persist



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for so long in the surface layers as on the heavier land. When the plots were first tested in October 1934, after a drag harrowing in September, there was no appreciable difference in resistance on the gyrotilled and control plots down to a depth of 6 in. There was, however, a highly significant difference in the subsoil, due to the breaking up of the pan on the gyrotilled plots. This latter effect is of a fairly persistent nature. It was scarcely significant when the plots were tested in May 1935, owing to the high errors caused by the stones in the hard dry ground. When rain had softened the soil again, the September test, 13 months after gyrotilling, showed that there was still a very significant difference in the resistance of the subsoil on gyrotilled and control plots. It is probable that this effect will persist for several years.

Table VI. *Dry Field. Table showing mean resistances (lb.) to penetration of probe in September 1935, 1 month after gyrotilling*

Depths in.	Gyrotilled	Ploughed and subsoiled	Ploughed
1	7.1	7.2	7.6
2	7.7	8.2	7.7
3	9.1	8.7	7.7
4	10.4	8.8	8.6
5	13.6	9.1	10.0
6	12.9	10.0	14.8
7	13.4	13.3	22.6
8	12.9	14.2	40.7
9	14.1	15.6	41.3
10	18.3	26.4	55.7
11	20.4	30.9	63.2
12	27.4	36.3	73.6

*Significance of differences.* From 7 to 12 in. resistance on ploughed plots was greater than that on both the others ( $P < 0.01$ ). There was no significant difference between gyrotilled and subsoiled plots.

### *Discussion of Table VI*

The significant feature of this table is the approximation of the resistance of the lower layers of the subsoiled plots to the low value of the gyrotilled plots. The soil of Dry Field is similar to that of Bunkers' Field, with a hard pan commencing at a depth of about 8 in. On Bunkers' Field in the previous year, the horse subsoiler which was used produced little impression on the pan. It is fairly certain that the results of subsoiling would have been similar on Dry Field if it had been attempted in August, when the land was dry and very hard. The gyrotiller broke into the pan only with great difficulty, and the horse subsoiler would probably not have penetrated it at all. Under the much softer conditions of September the subsoiler did useful work, and it is probable that the

gyrotiller would have worked to better effect at this time. Apart from the difference in the effect of subsoiling, the results of the tests confirm the work done on Bunkers' Field.

## V. SIEVING TESTS

### *Girton Allotment, March 1935, 20 months after gyrotilling*

In the preparation of the seed bed for spring oats, this field, which had been ploughed the previous October, was cultivated three times by four-horse teams pulling Ransomes' "Orwell" cultivators, fitted with spring-mounted tines. It was noticed that the furrow slices on the gyrotilled plots worked down more easily than those on the controls, and the difference was well demonstrated by a method of sieving similar to that described by Keen (6).

The sieves used were 20 in. in diameter and 3 in. deep. They have wooden sides and are fixed in a "nest" by means of three wooden slats screwed at equidistant intervals along the outside edge of each, and projecting about 3 in. over the next sieve below. The dimensions of the mesh are those suggested by Keen, viz.:

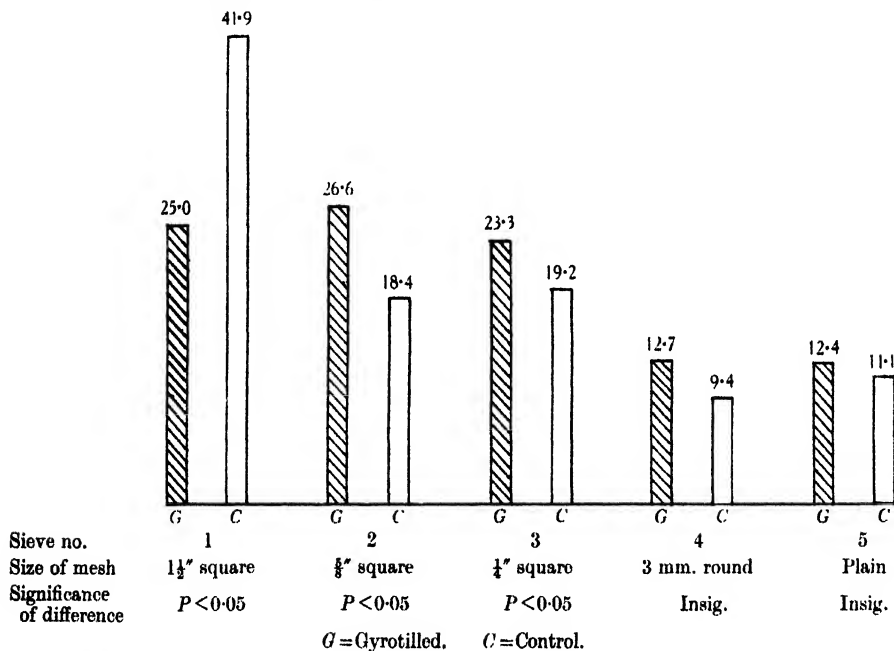
- (1) Top sieve: wire with  $1\frac{1}{2}$  in. square holes.
- (2) Second sieve: wire with  $\frac{5}{8}$  in. square holes.
- (3) Third sieve: wire with  $\frac{1}{4}$  in. square holes.
- (4) Fourth sieve: galvanised iron, with 3 mm. diameter round holes.
- (5) Lowest has a plain galvanised iron bottom.

The nest of sieves was constructed very cheaply. The top three are standard productions and the bottom two were easily made from standard materials.

### *The method of sampling*

A piece of  $\frac{1}{16}$ -in. sheet steel, 2 ft. long and 4 in. wide, was bent into a square frame, with sides 6 in. long and 4 in. deep, and welded at the junction. One edge of the steel was sharpened all round. This sharp edge was pushed into the soil until the upper edge of the frame was level with the soil surface. The surrounding soil was then cleared away at one side and a flat spade was pushed beneath the lower edge of the frame. The spade thus formed the bottom of a rectangular box full of soil, and this was carefully lifted out, any soil adhering to the edges removed, and the box of soil gently inverted on the top sieve of the nest. A composite sample was obtained from each plot by bulking three such samples taken at random.

The sieves were then lifted by two persons, who then shook them to and fro ten times. They were knocked against the shoulders of the two experimenters alternately at the end of each stroke. It was possible by this method to produce a shaking effect which was fairly constant from test to test. The method of sampling and shaking is a matter of convention in a test of this kind, but it is important to keep it uniform



Text-fig. 1. Figure showing relative proportions of particles of various sizes on gyrotilled and control plots 20 months after gyrotilling.

throughout the experiment. After shaking, the soil from each sieve was weighed and the fractions expressed as a proportion of the sample. The results are presented in Text-fig. 1.

#### *Discussion of Text-fig. 1*

It was noticed when taking the samples that on the control plots a wet mass of clay generally filled the bottom of the sampling frame. On the gyrotilled plots the tilth was deeper. A few wet clods were generally present in the lower parts, but there was not a solid wet layer such as was usually found on the controls. The result of this difference is seen in the significantly higher proportion of the first fraction on the control plots. The higher proportion of large clods on the control plots neces-

sarily implies a lower proportion of the smaller fractions. Too much emphasis should not, therefore, be laid on the relative proportions of any one fraction. The fact which Text-fig. 1, in conjunction with the observations made while sampling, brings out quite clearly is that there was a significantly deeper tilth on the gyrotilled plots. This was coupled with a better germination and early development of the crop on those plots.

## VI. MOISTURE CONTENT AND PERMEABILITY TESTS

It was hoped that the action of stirring up the subsoil by means of the gyrotiller would assist drainage on the heavy land by facilitating the rapid passage of the water into the mole drains. Nicholson(7) has pointed out the existence of indications that gyrotilling has achieved this desirable object. Direct evidence of the influence of cultivations on drainage is difficult to obtain. Drainage does not necessarily reduce the moisture content of the soil. Indeed, it has been pointed out by Russell(8) that moisture contents are frequently higher on a drained soil than on an undrained one.

The gyrotilled and control plots on all the experiments have been sampled for moisture content from time to time, but no significant differences have been demonstrable, though it was clear that the soil was behaving in a very different manner with respect to factors affecting drainage on the two series of plots.

Evidence concerning the drainage properties of the soil on Girton Allotment was obtained by means of permeability tests by a method similar to that of Flodkvist (quoted by Russell(8)). Sheet iron cylinders,  $3\frac{1}{2}$  in. in diameter and open at both ends, were forced into the soil on the plots to a depth of 2 in. A measured quantity of water was quickly poured into the cylinder, and the time taken for the level of the water in the cylinder to fall 3 in. was recorded. Five such readings were carried out in random positions on each plot, and the plot means were subject to statistical analysis. In a test carried out in November 1933, 4 months after gyrotilling, the mean time taken on the control plots was 5.6 times that on the gyrotilled plots. There was a very high error, the standard error of one plot being 55 per cent. of the general mean of plots, but the difference was so great that it was highly significant ( $P < 0.01$ ).

## VII. SUMMARY

A general account is given of four experiments in which the action on the soil of the Fowler "Gyrotiller" has been compared with that of the traditional cultivation implements. The gyrotiller has produced well-marked changes in the soil on both heavy and light land. These changes have been demonstrated and recorded by tests of the compactness, the resistance, the degree of comminution and the permeability of the soil.

On the heavy land, the initial effect has been to produce a very loose and open soil. This has gradually settled down until, after the end of the first winter, no difference in compactness was demonstrable by means of the revolver test. But the resistance of the soil, measured by a special recorder and by plough drawbar tests, has remained demonstrably lower on the gyrotilled plots than on the controls for a considerable time after this, and a method of soil fixation has shown that small cavities persisted for more than a year. In one experiment, differences in resistance have been shown to exist up to 19 months after gyrotilling.

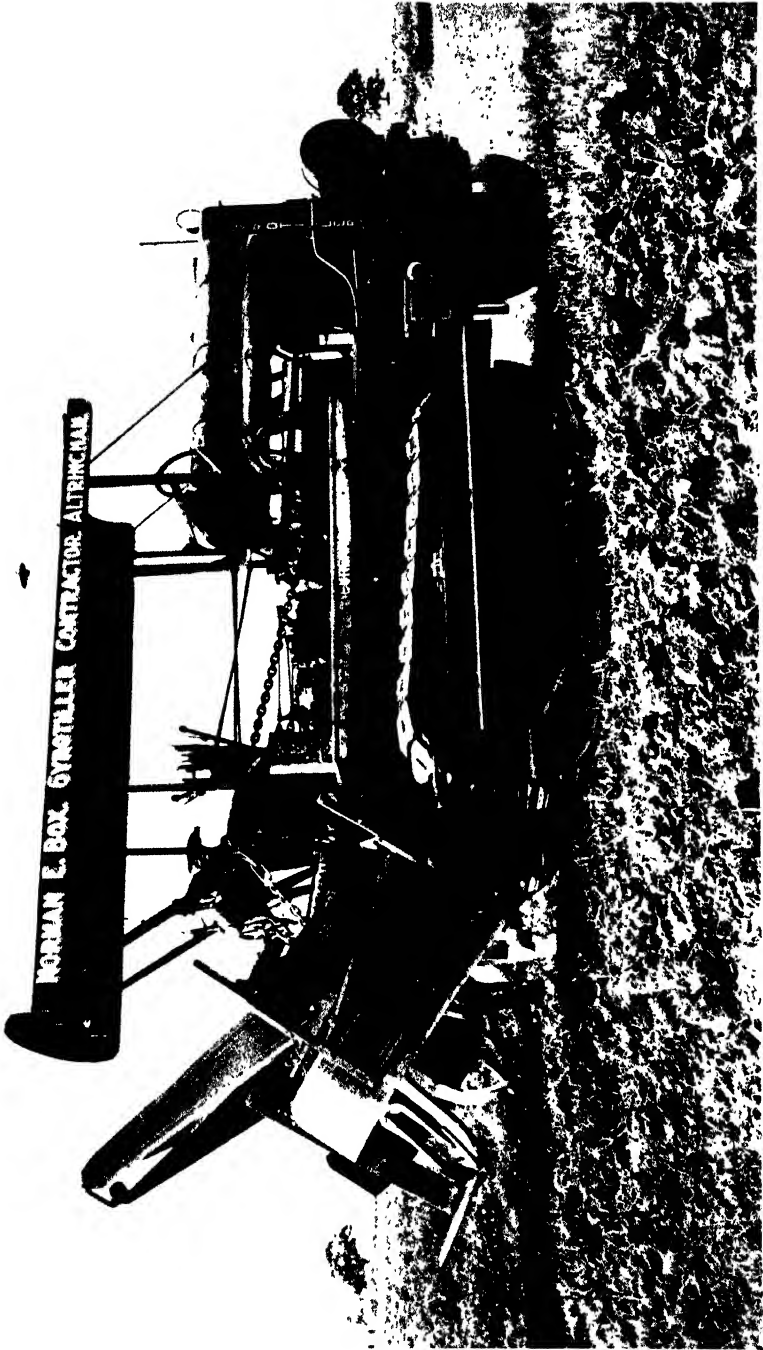
On the light land, a similar initial looseness has been produced, but this has not been as persistent as on the heavy land. The chief effect of gyrotilling on the light land has been the thorough breaking up of a pan which existed just below the ordinary depth of cultivations. The loosening of the subsoil shows no signs of disappearing after 13 months.

This work has been done in collaboration with Mr W. S. Mansfield, M.A., Director of the University Farm, Dr H. G. Sanders, M.A., and Mr F. H. Garner, M.A. My thanks are due to them and to Prof. F. L. Engledow, C.M.G., M.A., for help and advice. I wish also to acknowledge my indebtedness to certain graduate students, especially to Mr D. G. Bullard, B.A., Mr A. C. Owers, B.A., and Mr J. M. Watson, B.A., for assistance in part of the experimental work.

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(Received October 14, 1935)







Control plot, clean-cut furrow wall and bottom



Gerotilled plot, crumbled furrow wall and bottom





## SOILS IN RELATION TO MARSH SPOT OF PEA SEED<sup>1</sup>

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(With Three Text-figures and One Map)

MARSH SPOT, a defect found in seeds of green peas (*Pisum sativum* L.), has been known for many years in this country. It consists of one or more pockets of dead tissue at or near the centre of the cotyledons (Fig. 1). These are visible, as a rule, only on the inner surfaces. Occasionally the plumules are also necrotic. Marsh Spot first appears when the seeds reach their full size, *i.e.* at about the time at which they would be picked for cooking. It is first visible as one or more minute spots of a darker green, translucent or water-soaked appearance. The defect develops rapidly and the tissue of the affected area dies, becoming pale brown in colour. In some crops affected seeds occur indiscriminately in the pods among the healthy ones, but in others the diseased seeds are invariably those nearest to the stalk.

Marsh Spot is recognised by a number of seedsmen and farmers as being particularly prevalent among crops of peas grown on low-lying land near the sea coast. Romney Marsh in Kent and Foulness Island in Essex are two such areas, which are known to produce severely affected crops. Both have been reclaimed from the sea in comparatively recent times. It is probably owing to the association of the trouble with this type of land that the name "Marsh Spot" has had its origin. Severely affected crops are also grown on the low-lying polder lands of Holland. In England it has been reported also from districts remote from the sea and on soils other than those reclaimed from the sea, but until recently such observations had only been occasional. Owing to the bad reputation of certain districts for producing crops affected with Marsh Spot, some seedsmen have ceased to place contracts with growers in these areas. Others have suffered considerable financial loss from being unable to sell heavily affected seed received from the growers. Thus the disease has

<sup>1</sup> A grant to assist the work described in the present paper was received from the Ministry of Agriculture and Fisheries in 1933 and 1934.

involved farmer and seedsman alike. In spite of the fact that Marsh Spot is encountered in processed canned peas there appears to be little evidence as yet of its being regarded as serious in the canning industry.

Tests were carried out by the present writers, both in the laboratory and in the field, to observe the effect of Marsh Spot upon germination. The amount of Marsh Spot in the seed employed was ascertained by a statistical examination of other peas from the same sample. This method was employed as being more satisfactory than that of removing the seed

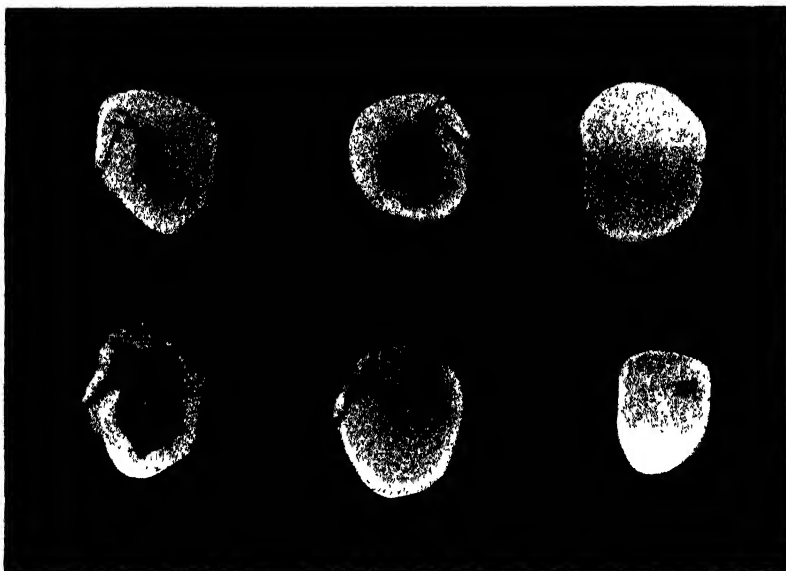


Fig. 1. Types of Marsh Spot. Inner surfaces of cotyledons.  $\times 2$ . Top row: (1) Typical. (2) Spotted. (3) Slight. Bottom row: (4) Advanced. Plumule badly necrosed and epidermis ruptured. (5) Peripheral lesion. Plumule unaffected. (6) Plumule only affected. Cotyledon healthy.

coats of the actual peas to be used for the germination test, and thereby attempting to find out the internal condition of each seed. Samples, heavily affected with Marsh Spot, gave quite satisfactory germination figures. A sample of Harrison's Glory for example, with 100 per cent. of the peas affected and with every plumule necrosed, showed a 95 per cent. germination. Where lesions were confined to the cotyledons, Marsh Spot appeared to have little effect upon germination, but, in cases where the plumule was involved, normal development was interfered with. In these the primary shoot elongated but ceased after a few days if the growing point was necrosed; laterals were then produced in the axils of

its embryonic leaves (Fig. 2). In the most severely affected cases, where the entire plumule was necrotic, the primary shoot elongated into a "blind" stump. Laterals then developed in the axils of the cotyledons. Appearance above ground was thus slowed down considerably and in the field the plants from such seed started irregularly (Fig. 3). Once established, however, their growth was quite satisfactory and the crop good. Nevertheless, in some cases, Marsh Spot has been found by other



Fig. 2. Germination—15th day. Production of lateral shoots. Nat. size. Left: Tip of growing point of main axis necrotic. Lateral shoot from axil of plumular leaf. Lateral shoots from axils of cotyledons beginning to develop. Right: Growing point of main axis completely necrotic. Lateral shoots from axils of cotyledons.

workers(1, 4) to be responsible for bad germination figures, and this adverse effect is mentioned by Pethybridge (3), p. 843).

A number of workers in England and Holland have shown that the disease is not transmitted through the seed, heavily affected crops being obtainable from clean seed and clean crops from heavily affected seed. In fact all available evidence points to a non-parasitic origin of the disease. Efforts to isolate an organism, or to produce Marsh Spot by inoculating plants with cultures from affected seed, have consistently given negative results.

The most recent account of an investigation of the causes of Marsh

Spot is that by Miss M. S. Lacey<sup>(1)</sup>, who deals with the bacteriological aspect, the germination and growth of seeds, the yields from affected and healthy seed, and the effect of soil and physiological conditions in pot experiments upon the production of Marsh Spot. Dr B. J. Grieve<sup>(2)</sup> has contributed a note on the microscopical investigation of affected seeds. A full account of the disease and a complete review of the literature has recently been given by Dr G. H. Pethybridge<sup>(3)</sup>. In Holland Miss H. L. G. de Bruijn<sup>(4)</sup> has described extensive plot experiments and incidentally reported the occurrence of Marsh Spot in France. The disease



Fig. 3. Seedlings growing in the field at Wye showing "stumped" plumule and basal shoots. 19th day of growth. Nat. size.

has now been recorded by Wade and Zaumeyer<sup>(5)</sup> in peas grown in California.

The writers of the present paper considered that it would be profitable to pay attention to the occurrence of the disease in crops of peas grown on a field scale, a study which seems to have been hitherto neglected in this country. With the aid of a grant from the Ministry of Agriculture an extensive survey was begun in the spring of 1933, of the incidence of Marsh Spot in crops of peas grown in Kent under contract for seedsmen.

From information supplied by seedsmen and farmers it soon became evident that they had known of the occurrence of Marsh Spot for a very long time, and a number of hypotheses were advanced by them as to its cause. The majority attached responsibility to soil conditions or to the weather, or to a combination of both of these. It was widely believed that wet weather, especially at harvest, resulted in a high incidence of Marsh Spot. Some, however, asserted that defective drainage of the soil

was the primary cause, while others suggested that affected peas might be more plentiful in those pods which were kept moist by lying in contact with the ground and shaded by the foliage. There was a strong belief that Marsh Spot was found only in land reclaimed from the sea, and many attributed this to the presence of common salt in the soil. It seemed advisable, therefore, to explore, in so far as might be possible, the effect of these factors upon the pea crop.

To examine the influence of the soil upon Marsh Spot the soils of as many fields of peas as possible, both in the reclaimed land of Romney Marsh and inland throughout east Kent, were surveyed and classified. At harvest samples of the crops were collected and examined for Marsh Spot. In this work the same procedure was followed in 1933 and 1934. Wherever it was possible samples were obtained of the seed sown in each field. A part of each of these was examined, to determine whether Marsh Spot was present in the original seed. The remainder was sown, a row to each sample, in a field on the College farm at Wye. In 1933 each row was divided into four parts, two of which served as controls while each of the others received a different treatment. In the two control sections the plants were allowed to trail on the ground, in accordance with the conditions of ordinary commercial cultivation. The first treatment consisted of growing the peas on sticks, so that none of the pods should lie in contact with the ground, and in the second, common salt was added to the soil. In 1934 likewise, a row of seed was sown from each sample, and again each row was divided into four parts. Two of these were dressed with sulphate of potash and the other two kept as controls. None of the rows was provided with sticks, and the treatment with common salt was not repeated. The application of potassic manure was prompted by the findings of Miss H. L. G. de Bruijn<sup>(4)</sup>, who showed that a reduction in the amount of Marsh Spot had resulted in plots to which potash had been applied, whereas there had been an increase in those to which a balanced manure had been applied. Still further to test the effect of potash upon the disease, five sets of plots, each of which contained four replications, were laid out on fields of peas in Romney Marsh.

The crops from the plots at Wye and on Romney Marsh were harvested and the peas examined for Marsh Spot, in the same way as the samples from the fields. The results are discussed on pp. 77-79.

## THE FIELD SURVEY

Each farm on which it was known that contract seed crops were being grown in 1933 and 1934 was visited twice in the spring or early summer, on the first occasion to collect a sample of the seed, and on the second to make an examination of the soil on which the crop had been sown and to take samples for chemical analysis. On both of these visits all possible data were collected which might have some bearing on the origin or incidence of Marsh Spot, viz. the date of sowing of the peas, any manures applied, the cropping and manuring practised for a few preceding years, and the history of the fields especially with regard to drainage, both natural and artificial.

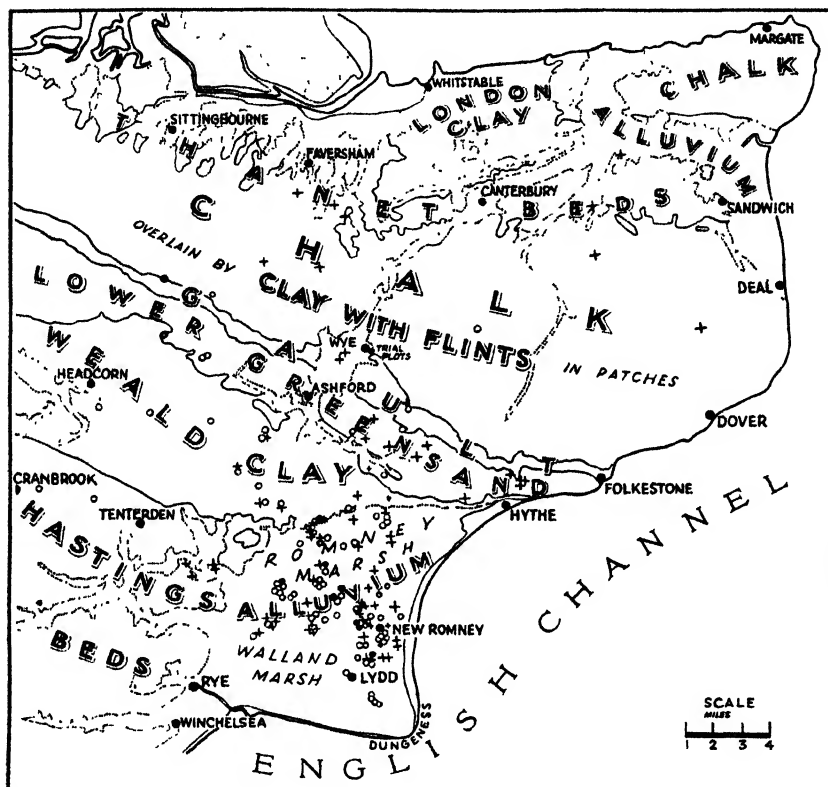
The method of surveying employed was the American System which has been described by Lee<sup>(6, 7)</sup> and by others. Under this system, the soils were identified in the field and grouped into "series". The great majority of those encountered belonged to series with which the writers were familiar, and of which descriptions have already been published. During the examination of the soils special attention was paid to the water circulation of each, and for this purpose borings were made to a depth of 4 ft. 4 in. (131 cm.).

The fields surveyed lie in the eastern half of Kent and cover a great variety of geological formations (Map 1). Commencing with the oldest, these are the Hastings Beds and Weald Clay (Wealden), Hythe Beds and Folkestone Beds (Lower Greensand), Gault, Chalk, Thanet Sand, Clay-with-flints, Valley Brickearth and Alluvium. The countryside is very varied, comprising the hills of the Hastings Beds, Lower Greensand and Chalk Downs, the low-lying country of the Weald Clay and Gault and the broad, flat expanse of Romney Marsh, much of which is below the level of the high tide. The relation of the geology to the landscape and farming in this area has been fully described by Hall and Russell<sup>(8)</sup>.

Out of a total of 167 fields surveyed 110 were situated in Romney Marsh. It is therefore advisable to describe this part of the area in greater detail.

Romney Marsh, comprising 41,000 acres including some of the finest agricultural land in the country, has been built up, during geologically recent times, by the combined action of the River Rother and the English Channel. The exact details of the very complicated process described by Gilbert<sup>(9)</sup> are still somewhat imperfectly known. The materials brought down by the river have been derived almost entirely from the fine sands and clays of the Hastings Beds, while the sea has

contributed a variety of materials from the same and other geological formations. Among these are the coarser sands and shingle, together with quantities of marine shells. The soils, classified and described by Cole and Dubey in the course of a survey of Romney Marsh pastures (10),



Map 1. Marsh Spot of pea seeds. Map of east Kent, showing distribution of fields surveyed in 1933 and 1934 and their relation to the geology.

- Position of a field with seed pea crop, examined 1933.
- + " " " 1934.
- ⊕ " " " 1933 and 1934.

show easily recognisable profiles, more especially in the matter of texture. A water table is present throughout Romney Marsh, its depth from the surface varying from place to place. In the fields surveyed the water table was usually to be found between 2 and 5 ft. below the surface. Thus in the majority of borings water flowed into the hole, and after a short space of time reached the level of the water table, *i.e.* the level



to which the ground is saturated with water. The depth of the water table below the surface fluctuates in accordance with prevailing climatic conditions but is, to a certain extent, controlled artificially by pumping the water out or holding it back as the season demands. The rate of percolation of rain water from the surface of the soil down to the water table varies considerably according to the nature of the soil; little of the land, however, could be described as badly drained.

The growing of contract pea-seed crops was encountered on five soil series in Romney Marsh, and these are briefly described below. Fuller descriptions of these soils under pasture conditions have been published by Cole and Dubey (10).

*New Romney series.* The texture of the soil is light and sandy throughout. The surface soil, fine sandy loam to loam, is sometimes as deep as 24 in. On freshly broken pasture its colour is a dark grey-brown owing to the presence of large quantities of organic matter, but on older arable land it is usually a rather pale brown. Below this surface layer there is sometimes a slight stiffening in texture, but it is never heavier than a silty loam. From this horizon downwards the soil grows progressively lighter, passing into a sandy loam or loamy sand of yellowish brown colour. Soils of this series vary greatly in depth, and shallow phases were not infrequently recorded. Typically the natural drainage is good, although in a few cases the water table was close to the surface and the depth of well-drained soil was consequently small.

*Finn series.* The soil texture, which at the surface is loam or silty loam, stiffens until at 24–36 in. it becomes silt loam or silty clay loam. Below this it lightens once more, often very abruptly, and passes to sandy loam or loamy sand. The surface soil is generally a deep rich brown, which becomes paler and lightly mottled and iron-stained in the horizon of heaviest texture. Below this the colour is yellowish brown. The drainage is free in the first 2 ft. but at this point is slightly impeded.

*Brenzett series.* As in the *Finn series* the texture stiffens from the surface downwards, and then grows lighter again. In this series, however, the texture generally is heavier throughout. The surface silt loam passes into silty clay loam or clay loam which then gives place to silt loam deeper still. The surface layer of soil is shallower than in the *Finn series* and the mottling in the horizon of heaviest texture is more pronounced, thus indicating less free drainage. The lower layers of the soil are bluish grey in colour.

*Ivychurch series.* The soils of this series are heavy but, at a depth of about 3 ft. the texture becomes markedly lighter. The silty clay loam

of the surface stiffens deeper down to clay loam, which persists until the lighter material, already mentioned, is reached. The texture of this last is fine sandy loam or even loamy sand. The surface soil is generally fairly dark grey-brown in colour which becomes mottled and iron-stained at about 18 in. as a result of impediment of the drainage at this point.

*Newchurch series.* These soils are heavy throughout their entire depth. The surface texture of silty clay loam gives place to clay loam which persists to a depth of more than 52 in. The surface layer, some 8–14 in. deep, is a fairly dark grey-brown, which gives place below to a mottled yellowish brown and blue-grey material. Drainage is poor below 18 in. and, in consequence, there is iron deposition at this point.

Lime is plentiful in the lower layers of all the soils of Romney Marsh, white, calcareous concretions being common in the heavier textured examples. The reaction of the surface horizons, on the other hand, is found to vary. In the majority of cases it is alkaline, but acid examples are found in all except the *Newchurch series*. Acid conditions are most frequent and most strongly developed in the soils of the *New Romney series*.

The soils encountered outside Romney Marsh were grouped in twenty-four series, presenting a great variety of soil conditions. These are very briefly described below, in the geological order of their parent materials and taking the oldest first.

On the Hastings Beds peas were grown on four soil series. The first three were derived from the Tunbridge Wells Sand division: the *Pembury series* of well-drained sedentary soils, the *Curtisden series* of poorly drained sedentary soils, and the *Teise series* of poorly drained, deep, transported soils. In the *Hartley series* the material derived from Tunbridge Wells Sand gives place to Wadhurst Clay within 42 in. In all these the typical texture is very fine sandy loam, which in the case of the *Hartley series* gives place to clay loam. The surface soil was faintly acid in each case, but the reaction was variable deeper down. These soils have already been described by one of us (11).

Peas were fairly extensively grown on the soils of the Weald Clay. The *Hildenborough series* and the *Thorne series* both consist of sedentary soils, the former being well drained and the latter badly drained and showing signs of periodical waterlogging. Both are heavy in texture, passing from silt loam or silty clay loam at the surface to clay loam below. All the examples surveyed were strongly acid throughout. These soils were briefly described by Lee (7). Six examples were also recorded of a fairly well-drained alluvial soil derived from Weald Clay. The texture

passed from silt loam at the surface to silty clay loam below and the reaction was acid. One of these showed a water table at a depth of 36 in. in June 1934. These soils have not yet been named nor descriptions published.

On the Lower Greensand six soil series derived from the Hythe Beds were surveyed. In the *Willington series* the surface loam gives place to a bright red-brown sandy clay loam. The *Sutton series* consists of a surface loam which passes into an orange-brown silt loam, showing weathering seams of limestone. The soils of the *Medway series* consist of deep, uniform well-weathered drift materials derived from the Hythe Beds. The *Elmstone series* consists of mixed Hythe Beds materials of various degrees of weathering accumulated as a result of landslips. All these series are well drained except occasional wet patches in the *Medway series*, due to springs. Generally speaking the *Willington* and *Sutton series* are acid and the remainder alkaline.

The Folkestone Beds provided two series: the *Bearsted series* consisting of a shallow, eroded soil of loamy sand texture, and the *Lowland series* of uniform, deep, colluvial soils of sandy loam texture. The soils of the Lower Greensand have been fully described by Bane and Gethin Jones (12).

On the Gault a field was surveyed on the poorly drained, sedentary *Broadway series* (Lee(7)). The *Sharbrooks series* gave a heavy alluvial Gault soil containing some chalk fragments. The drainage was poor and a water table was present in a part of the field. The reaction of both series is alkaline (Brade-Birks and Furneaux (13)).

The Chalk was represented by two series, both composed of fluvio-glacial drifts. The *Coldharbour series* has a loam surface soil which passes into a chalky Coombe Deposit, and is very well drained. The *Brook series*, that on which the plots were laid out on the College Farm, consists of a structureless pale grey, highly calcareous Coombe Rock. A water table is present in this series (Brade-Birks and Furneaux (13)).

On the Thanet Sand an example of the sedentary, well-drained *Hall series* was surveyed (Lee(7)).

The Clay-with-flints provided a number of examples of the well-drained, sedentary *Rattle series*, in which a brown loam surface soil passes into a deep red, clay loam subsoil containing numerous flints. The drainage was good and the reaction acid (Lee(7)).

On the Valley Brickearth eleven examples of deep, well-drained reddish brown loams were surveyed, belonging to the *Wye series* (Brade-Birks and Furneaux (13)), and one example of the closely related *Langley*

*series* (Bane and Gethin Jones (12)). Three examples of brickearth showed signs of poor drainage in the lower layers of the soil and thus belong to the *Brices series* (Brade-Birks and Furneaux (13)).

#### SAMPLING OF THE CROPS AND EXAMINATION OF THE SEED

As each crop of peas ripened a sample of the pods was taken from it. In fields where the peas were growing on more than one soil series a separate sample was gathered from each soil area. The pods were collected as the crop was being cut or very shortly afterwards and before any of the cut haulm had been moved. In 1933 the harvest period extended from July 13 till August 18, and in 1934 from July 7 till August 7.

The method employed was to pick a handful of pods at each of a number of places scattered as evenly as possible throughout the area to be sampled, and for each sampling a bag containing roughly 6 litres of pods was filled. Care was taken to make each picking as representative as possible by gathering large, medium and small pods, ripe and unripe pods, pods lying beneath the bine upon the ground, and pods hanging clear of the bine, pods borne near the top of the plant and those from near the base, in numbers proportional to their presence in the crop. The samples were taken to Wye and spread out to dry in shallow layers on the staging of a glasshouse. The pods were stirred from time to time and when completely dry were stored in paper bags. Threshing was done by hand and the seed was stored in paper bags.

For the purpose of examining each sample for Marsh Spot a quantity<sup>1</sup> of the seed was soaked in water for 48 hours. The peas were then placed in a single layer in a Petri dish, in order to give each an equal chance of being selected. A total of 100 peas was picked from the dish at random, ten at a time. The seed coat of each was removed and the cotyledons separated and examined. At the same time the plumule was carefully scrutinised. After recording the number of healthy and affected peas in each group of ten,  $\chi^2$  was calculated, and if this exceeded 16.919 the sampling error was considered too large and more peas were soaked and an additional fifteen groups of ten were examined. The corresponding value of  $\chi^2$  for the larger number of peas was 36.415. In only one or two instances was it necessary to examine more than 100 peas, and where this was done, the sampling error always fell within the limits chosen.

<sup>1</sup> About 250 peas were taken from the bag in such a manner as to include seed from the top, centre and bottom. Those obviously damaged by insect or fungus attack were discarded.

THE DISTRIBUTION AND INTENSITY OF MARSH SPOT  
ON THE SOILS SURVEYED

During the two years 165 fields of contract peas were surveyed in detail, totalling approximately 936 acres (see Table I). Owing to the presence of more than one soil series in many of the fields, a total of 229 samples of peas was collected from the field crops. In both years a large number of the crops was found to be severely affected with Marsh Spot, and in several cases 90 per cent. or more of the peas were spotted.

Of the 109 field samples collected in 1933, seventy-five were from Romney Marsh and thirty-four from other parts of the county. Of the Romney Marsh samples, sixty-four were affected with Marsh Spot ranging in intensity from 1 to 91 per cent., while of samples from elsewhere nine were affected, the intensity ranging from 1 to 8 per cent. From the trials on the College farm at Wye 165 samples were harvested, and of these 117 were affected with Marsh Spot, ranging from 1 to 52 per cent. In 1934, 120 field samples were collected; sixty-five from Romney Marsh and fifty-five from elsewhere. Of those from Romney Marsh forty-five showed Marsh Spot ranging from 1 to 98 per cent., while those from elsewhere showed only four affected samples, in which the heaviest incidence was only 2 per cent. From the trials at Wye 306 samples were harvested; 157 of these showed Marsh Spot, the intensity ranging from 1 to 36 per cent.

Table I. *Distribution and intensity of Marsh Spot*

Year	Locality	No. of fields surveyed	Area acres	No. of samples of seed	No. of samples affected	% samples affected	Highest inci- dence %
1933	Romney Marsh	56	352	75	64	85.3	91
	Elsewhere	26	103	34	9	26.5	8
	College farm	1	Plots	165	117	70.9	52
	Total	83	455	274			
1934	Romney Marsh	51	290	65	45	69.2	98
	Elsewhere	32	191	55	4	7.3	2
	College farm	1	Plots	306	157	51.4	36
	Total	84	481	426			
Grand total		167	936	700			

In the two years under review the incidence of Marsh Spot, as shown in Table I, was very much more severe in Romney Marsh than throughout the rest of the county in both the number of crops affected and the percentage of damaged seeds in affected samples. Of the peas grown

outside Romney Marsh by far the most heavily affected samples were to be found among those grown in the trials on the College farm. In fact, in a number of cases, a considerably higher percentage of Marsh Spot was found in the samples from Wye than from the corresponding crop grown in Romney Marsh from the same stock seed.

The examination of the distribution of Marsh Spot in Romney Marsh showed that in both seasons there was a close general relation between the soil upon which the peas were grown and the severity of the disease. Although crops free from Marsh Spot were found on each of the five soil series on which peas were grown in the Marsh, the number of affected crops and the average severity of the disease were found to be greater on some soil series than on others. Table II shows the incidence of the disease in relation to soil series in Romney Marsh.

Table II. *Percentage of Marsh Spot on five soil series in Romney Marsh*

Soil series	1933 crop		1934 crop	
	% peas affected (average of all samples)	No. of samples	% peas affected (average of all samples)	No. of samples
New Romney	5.05	19	4.36	22
Finn	7.16	12	14.64	14
Brenzett	15.29	7	27.75	12
Newchurch	25.86	21	35.90	10
Ivychurch	30.22	9	29.50	10

In calculating the above figures for the 1933 crop, the results from two fields have been omitted owing to the remarkably high incidence of Marsh Spot which they exhibit when compared with the other crops of the same soil series. In the *New Romney series* the nineteen crops upon which the figure of 5.05 per cent. is calculated showed a range of 0–21 per cent., whereas the one exceptional example carried five crops in the one field which showed a range of 70, 74, 83, 85 and 91 per cent. of affected peas. Similarly in the case of the *Finn series* the twelve crops on which the figure of 7.16 per cent. is based showed a range of 0–22 per cent., while the one exceptional field bore a crop with 69 per cent. of affected seed. The difference between the crops on these two fields and the rest of the crops on the same soil series appeared to warrant their separation, when calculating the average incidence of Marsh Spot for the soil series. If, however, the crops from these two fields are included with the rest, the average incidence for the *New Romney series* is raised from 5.05 to 9.79 per cent. This figure is calculated upon the basis of the percentage

of Marsh Spot in the crop from each field, so as to overcome the undue influence which a field carrying five crops of peas would have, if each were included separately in determining the average for the soil series. Similarly, the figure for the *Finn series* is raised from 7.16 to 11.92 per cent.

When the examination of the pea samples revealed the very heavy incidence in these two cases, both fields were revisited and carefully examined and so also were all data concerning each. It was, however, impossible to detect any factor to which so great a difference could be attributed. Certainly in the case of the field on the *New Romney series* the water table was high, but there were other fields on the same soil series with equally high water tables, which failed to produce such heavily affected crops. No explanation can be advanced to account for these two remarkably severe cases.

In the 1934 crop no such exceptional cases were encountered. It will be seen that when the soils are arranged in ascending order of the percentage of Marsh Spot found in the crops growing upon them (as in Table II), the order, except in the case of the closely related *Newchurch* and *Ivychurch series*, is the same in both 1933 and 1934. The survey being one of commercial crops involving a large number of varieties, their distribution among the soil series was accidental, and it was not possible to find any one variety on all the soil series. It is reasonable to suppose, however, that any possible varietal influence may largely have been counteracted by the number of cases investigated. The general agreement in the results of the two years' work appears to confirm this.

In twenty-six cases in Romney Marsh two soil series occurred within the boundary of one field, and one variety of pea was therefore grown on the two soils with other conditions as nearly as possible uniform. When the estimation of Marsh Spot had been made in the peas grown on the two areas, it was found that the order of the soil series in Table II was confirmed in ten cases, while in only one case was it reversed. In nine cases the difference was insufficient to be considered significant. In six other cases, however, where the *Newchurch* and *Ivychurch series* were found adjacent in the same field, the *Newchurch series* carried the more heavily affected crop in two cases and the less heavily affected crop in three cases, while in one there was no significant difference. In view of this and of their reversed positions in the average incidence of the disease in 1933 and 1934, it seems justifiable to regard the *Newchurch* and *Ivychurch series* as producing similar effects. Taking all the soil series the tendency is for the crops of peas grown on the *New*

*Romney series* to show the least Marsh Spot, those on the *Finn series* and *Brenzett series* an intermediate amount, and those on the *Newchurch* and *Ivychurch series* together the most.

It is interesting to note that this is the order in which these soil series fall if they are arranged according to their texture, the *New Romney* being the lightest and the *Newchurch* and *Ivychurch series* together the heaviest. At first sight this would appear to suggest that soil texture has a direct influence upon the incidence of Marsh Spot and that the heavier the soil the greater its tendency to produce Marsh Spot in the crop it carries. That this is not the case is conclusively proved by the examination of the peas grown on the *Hildenborough* and *Thorne series* outside Romney Marsh. Both these are very heavy Weald Clay soils, which closely resemble those of the *Newchurch* and *Ivychurch series*, themselves probably largely derived from Weald Clay materials. In twelve samples collected from the *Hildenborough* and *Thorne series* not a single pea was found affected with Marsh Spot.

Although the clay soils of Romney Marsh closely resemble those of the Weald Clay there is at least one important difference: all the soils of Romney Marsh possess a water table, while those of the Weald Clay do not.

#### THE RELATION OF A WATER TABLE TO INCIDENCE OF MARSH SPOT

The soils of the Marsh are alluvial and occupy flat, low-lying country; they are freely drained or fairly so, to a certain depth, below which the soil is saturated with water. The Weald Clay, on the other hand, is impervious to water and its soils therefore possess no water table; the country, though not hilly, is much more elevated and undulating than that in Romney Marsh. Contrary to popular belief, Romney Marsh is not a marsh in the true sense of the word. Most of the land is well drained, in fact far better so than much of the country on the adjacent hills. The realisation of this is the more important because Marsh Spot has been connected by some writers with wet, badly drained land. The *Thorne series* on the Weald Clay (outside Romney Marsh) is a thoroughly badly drained soil and yet, as already stated, not a single diseased pea was found among the eight samples grown on it. Further confirmation of this point is afforded by one crop which was grown on a field half on and half off Romney Marsh. The half lying off the Marsh sloped up a hill and consisted of a very badly drained alluvial soil showing signs of waterlogging in wet weather. The half on the Marsh was flat and consisted of an alluvial soil of similar texture though well drained, and with



a water table at 32 in. On examination of the crop grown on each half it was found that the peas grown off the Marsh were free from Marsh Spot, in spite of bad drainage, while those grown on the Marsh contained 6 per cent. of affected seeds.

Reverting to the consideration of the significance of the presence of a water table in the soil in relation to Marsh Spot, it seems possible that it exerts an influence by providing the plant with a plentiful supply of water throughout the growing period. The majority of the badly drained soils, on the other hand, such as the *Thorne series* where the soil is underlain by impervious Weald Clay, are badly drained owing to the inability of rain water to penetrate below the soil and soak away. Although such soils are wet during a rainy period, having but little reserve of moisture, they become very dry at other times. This was undoubtedly the case during the two dry summers of 1933 and 1934. The soils of Romney Marsh, by contrast, in those years remained quite well supplied with water by virtue of their water table within easy reach of the surface. It is true that large cracks opened in some of them, but at a little depth the soil was moist.

While the soils were being surveyed during May, measurements were made of the depth of the water table in the soil. A certain number of the borings did not reach the water table within 52 in., but in the great majority of cases it was encountered much closer to the surface than this. It was found at all depths from 24 in. downwards, and no fields were surveyed in Romney Marsh in which the water table throughout was below the 52-in. level. An intensive survey of the water table commonly showed considerable undulation of its surface, often within a short space and even though soil conditions appeared otherwise uniform and the ground flat. In one example two borings 20 ft. apart in the *Newchurch series* showed the water table at 36 in. and below 52 in. respectively. In another field on the same soil series borings 12 ft. apart encountered the water table at 38 in. and below 52 in. In 1934 further measurements were made in a number of cases early in July, for comparison with those taken in May. Very little rain had fallen in the interval (see Table IV), and in consequence the level of the water table had fallen somewhat. The amount of this fall was between 2 and 18 in.

The possibility was explored of correlating the intensity of the incidence of Marsh Spot in each soil series with the height of the water table in each case, but it was found that the presence of a large number of other factors such as variety of pea, system of manuring, and the length of growing period, introduced complications which made it impossible

to arrive at any conclusions. It was nevertheless evident that many of the most affected samples originated, in all the soil series, from fields in which the water table was closest to the surface. In the case of a field on the *Ivychurch series* the water table throughout the greater part was below 52 in., but in a piece about an acre in extent, the water table stood at 28 in. The crop from that part in which the water table was the deeper showed 5 per cent. of Marsh Spot, while that from the part with the water table at 28 in. showed 38 per cent. To quote a further example, two crops of different varieties were grown in 1934 on a field on the *Finn series* in which the water table was high (25-36 in.). The samples of peas from this field showed 62 and 33 per cent. of affected seed, as compared with 14.64 per cent. which is the average for all the crops on that soil series in 1934.

Outside Romney Marsh, in the course of the two years' work, peas were encountered growing upon only two fields in which a water table was present sufficiently close to be likely to have any influence upon the crop. In 1933 a crop growing on the *Sharbrooks series* showed 8 per cent. of Marsh Spot; the highest figure for a crop outside Romney Marsh, if the trials at Wye be excepted. This high incidence of the disease provoked a second and very close examination of the field. It revealed the presence of a water table in a narrow strip across the southern end of the field. The water table was 30 in. from the surface at its closest, but as the slope of the field was ascended this depth increased. Thus it was only for a small part of the distance that the water table was within the reach of a 52-in. auger. As this comparatively small area was not discovered until the peas had been harvested, only a single sample was taken from the whole field, and it is possible that the affected peas may have grown mainly upon this part, although the sample of pods was evenly drawn from all over the field. The crop throughout the whole field, however, was very green and leafy during the growing period and reached a height of about 2 ft. It happened that some of the same stock seed had been sown on the *Bearsted* and *Wye series*, only  $1\frac{1}{2}$  miles distant. Here there was no water table and the crop was completely free from Marsh Spot; it ripened about a fortnight earlier and produced a much less vigorous plant, reaching a height of only 9 in.

The second case was provided in 1934 by a field with alluvial soil derived from and overlying Weald Clay. In one corner of this a water table was discovered at a depth of 36 in. The peas from this part of the field were sampled separately from the rest, but the seeds from both areas of the field showed no Marsh Spot when they were subsequently

examined. The crop from the same stock seed grown in the trials at Wye also produced seed free from Marsh Spot, and it therefore seems likely that the variety was one not very prone to the disease.

It is significant that by far the most heavily affected crops, outside Romney Marsh, were those grown in the trials at Wye. Here as in Romney Marsh there was a water table close to the surface. At the beginning of the growing period it stood only 18 in. from the surface, but the level subsided gradually under the influence of the drought. The deep, calcareous, alluvial soil had until a few years previously been wet and boggy owing to the action of a nearby spring, but pipes had been laid and the ground rendered fit for cultivation. The spring, however, still supplied sufficient water to keep the soil very moist during the drought summers throughout which it carried the pea trials.

When the distribution of Marsh Spot is examined throughout all the crops grown in 1933 and 1934, both on and off Romney Marsh and in the trials at Wye, it is evident that all those severely affected were grown on fields with a water table within 52 in. of the surface. While these soils produced crops with up to 100 per cent. of the peas affected, the soils possessing no such water table produced a maximum of only 3 per cent. in a single instance. As has been indicated in Table I, a number of crops were encountered, which were free from Marsh Spot in spite of their having been grown on soils having a water table within 52 in. It nevertheless appears highly probable that a severe incidence of Marsh Spot is directly connected with the presence of a water table, close enough to the surface to provide a plentiful supply of water to the roots of the plants. The fact that Marsh Spot was most severe on the heavy soils and least so on the light ones in Romney Marsh, may probably be connected with the difference in conductive power offered to water by soils of different textures.

It might be thought possible, by means of pot cultures, to determine the relation of water table to soil moisture, or at least the direct relation of water table to incidence of Marsh Spot. In the opinion of the writers, the transference of soil, unless perhaps in the form of monoliths, so utterly changes its physical properties as to render the experiment useless. Merely to stand a pot of soil, or more correctly the surface horizon of a soil, in water held at a constant level is to reproduce water-logged conditions, rather than the well-drained state associated with the majority of cultivated soils possessing a water table.

## PLOT EXPERIMENTS AT WYE AND IN ROMNEY MARSH

On the trials at Wye (see p. 63) the peas of any one variety which were grown on sticks during 1933 gave a heavier crop than those which trailed on the ground, but all the fifty-five rows tested failed to show any significant difference in the amount of Marsh Spot present. Marsh Spot was plentiful both in the seeds from the stucked peas and from the controls. In spite of the drought the ground on which the pea bine lay was always moist, and so also was the bine lying in contact with it. It seems therefore that there is little foundation for the belief that external moisture from the soil has any direct influence upon the amount of Marsh Spot in the peas inside the pod.

The plots which were treated with common salt also gave a negative result. Salt had been added to the soil<sup>1</sup> at the time of sowing to test the supposition that Marsh Spot was prevalent in land reclaimed from the sea, owing to the presence of salt. There was, however, no significant difference between the peas from the treated portions of rows and those from the control portions. It is therefore concluded that Marsh Spot cannot be attributed to the presence of sodium chloride in the soil. This conclusion is strengthened by the analysis of samples of soil of the *New Romney series* from the field in Romney Marsh, which produced five exceptionally heavily affected crops. This soil showed only the faintest trace of chlorides. A similar observation has been made by van der Lek (14).

That Marsh Spot is not confined to land reclaimed from the sea is proved by the presence, on the plots at Wye, of crops showing up to 56 per cent. of affected seed. These plots were on land 10 miles from the coast, which has certainly not been beneath the sea since Pliocene times.

Generally, slight differences in the percentages of Marsh Spot were observed between the control rows, and those which were stucked, and those to which salt was applied, but these occurred irregularly and were mainly well within the sampling error. In a few instances the differences in the incidence of Marsh Spot in seeds from the treated and untreated parts of the rows were fairly large. In all such cases confirmation was obtained by the examination of more peas from these samples. In these examples it was observed that the sample showing the highest incidence

<sup>1</sup> At the time of sowing common salt (sodium chloride) was applied at the rate of 0.5 gm. per foot of row. From the time the peas were 3 in. high until harvest they were sprayed twice a week with aqueous salt solution, to simulate the salt content of the atmosphere in coastal districts. For the first month a strength of 0.13 per cent. was used which was increased to 0.3 per cent. thereafter.

of Marsh Spot was that which had the greatest proportion of large peas in it, and, as has already been noted by Miss de Bruijn (4), p. 287), more affected seeds were found among the large peas of a sample than among the smaller ones. A similar observation was made by us in 1934.

The plots laid out at Wye in 1934 to test the effect of potassic manure were treated with sulphate of potash (50 per cent.  $K_2O$ ) at the rate of 5 cwt. per acre, a few days before the peas were sown. On five fields in Romney Marsh plots were dressed with sulphate of potash at the same rate. In each field a block of eight plots was marked out, each  $1/50$  of an acre in area. On four of these the sulphate of potash was applied a few days after the seed was sown; the remaining four plots were kept as controls. In none of these, however, either at Wye or on Romney Marsh, was there any significant difference in the incidence of Marsh Spot on the treated and control plots. This result failed to confirm the observations of Miss de Bruijn (4)<sup>1</sup>, but this may perhaps be explained by the remarkably dry summer and the late application of the manure. Very little rain fell from the time the fertiliser was applied until after harvest and any effect was obviously minimised.

Table III

Plot trial	Soil series	Depth of water table		Control plots (4 in each trial) % Marsh Spot	Potash plots (4 in each trial) % Marsh Spot
		May 8 in.	July 3 in.		
1	Newchurch	28-36	38-52	100, 100, 100, 100	100, 100, 100, 95
2	Newchurch	27-30	36-40	100, 100, 97, 99	98, 99, 95, 96
3	Finn	42-52	44-48	7, 23, 5, 4	8, 4, 7, 27
4	Brenzett	24-30	41-42	23, 29, 28, 36	30, 20, 25, 21
5	New Romney	46	48-49	62, 18, 9, 45	46, 51, 7, 49

Although there was no significant difference between the treated and control plots in the potash trials in Romney Marsh, there was a remarkable variation (see Table III) in the incidence of Marsh Spot among the plots of the same treatment in two of the five trials (Table III, Nos. 3 and 5) although the soil was quite uniform. This may correspond to the uneven distribution of Marsh Spot recorded by Miss de Bruijn (4).

<sup>1</sup> Miss de Bruijn found that, although Marsh Spot was not a deficiency disease, it could be reduced appreciably by the application of potassic manures, provided these were applied alone and without any nitrogenous fertilisers. She attributed this result to earlier maturation produced by the potassic manure. Miss de Bruijn applied the potash as a double sulphate of potassium and magnesium (26 per cent.  $K_2O$ ) at approximately 8 cwt. per acre, i.e. about 2 cwt.  $K_2O$  per acre, while in the experiments described in the present paper the rate was  $2\frac{1}{2}$  cwt.  $K_2O$  per acre with only a trace of magnesium present.

It seems possible that the undulation of the surface of the water table, referred to above, may be connected with this phenomenon.

Four varieties of peas were employed, trials 4 and 5 being with crops from the same stock seed. Trial 5 was carried out on the field which, in 1933, gave the exceptionally high incidence of Marsh Spot on the New Romney series (see p. 71).

#### OTHER FACTORS IN RELATION TO INCIDENCE OF MARSH SPOT

##### (a) *Variety*

It is probable that varietal susceptibility to Marsh Spot exists, but in the present investigations it was found impossible to arrive at any conclusions, mainly because of the very large number of varieties<sup>1</sup> grown under contract in the fields surveyed.

Samples from the stock seed used in the contract fields were sown under uniform conditions in the trials at Wye, and the crops they produced were treated and harvested alike. Altogether forty-one samples of seed were sown side by side in 1933, and these were made up of twenty-eight different varieties. In 1934 seventy samples were sown, comprising thirty-seven varieties, but as twelve of these varieties had already been included in the previous years' trials, a total of fifty-three varieties was tested in the two years. The twelve varieties which were grown in both years' trials failed to show the same degree of susceptibility, relative to one another, during the two years. When they were arranged in ascending order of the severity of the disease in each year, varieties near the head of the list one year were near the foot of the list the next year and *vice versa*. However, all the samples of a particular variety from one seedsman behaved similarly during the same season, while samples of the same name from another seedsman often showed a considerable divergence. It therefore seems that, for the satisfactory analysis of the varietal factor, a more complete replication of samples of each so-called variety will be necessary and the experiment will have to be carried out over a longer period of years.

It was, of course, impossible to make comparisons between samples of any one variety grown on different fields, with a view to measuring varietal influence, because of the differences of soil, management and other factors which would be introduced. All observations on variety

<sup>1</sup> What constitutes a variety is, however, a difficult point to establish, as strains within a variety can vary in their practical importance sufficiently to make it necessary for a seedsman to send out his various strains under his own descriptive name, so that no definite record of varieties can be obtained.

had therefore to be based on the trials at Wye where all the peas were grown under uniform conditions.

In their examination of varietal susceptibility the writers were greatly assisted by seeds firms who placed at their disposal records covering a number of years. Owing to the lack of evidence as to the soils upon which the crops thus recorded had been grown, the information could be used as a rough guide only. One firm observed that round seeded varieties had been affected to only half the extent that wrinkled peas had been, and that generally the smaller seeded varieties in both groups, and particularly the early ones, had suffered to a less extent. This statement that early maturing varieties show, as a general rule, less susceptibility than the later ones is in support of the findings of Miss de Bruijn ((4), pp. 307 *et seq.*). The writers of the present paper, however, failed to establish any such relation between earliness of maturity or length of growing period and the incidence of Marsh Spot. On the trials at Wye, the earliest variety to ripen in 1933 showed 13 per cent. of Marsh Spot, while the last, which ripened three weeks later, was completely free.

The statement from the trade that the smaller seeded varieties are less susceptible than the large seeded ones assumed particular interest following Miss de Bruijn's observation that in any particular sample of peas the heavier seeds are more severely affected than the lighter ones ((4), p. 287). This observation was confirmed by the writers of the present paper (pp. 77 and 78).

#### *(b) Previous cropping*

The records of the previous cropping of the fields failed to show any correlation with the incidence of Marsh Spot. It is the common practice in Romney Marsh to grow one, two or even three successive crops of peas immediately after breaking up a pasture; while more than two-thirds of the crops examined in Romney Marsh were so grown, only a very few were encountered elsewhere. Where this practice was followed the amount of humus in the soil was, in many cases, very large, but it appeared to have little or no influence upon the amount of Marsh Spot in the crop.

#### *(c) Rainfall*

The belief, commonly held, that Marsh Spot may be directly connected with wet weather received little confirmation in 1933 and 1934 (see Table IV), when, with two remarkably dry summers, 85.3 and 69.2 per cent. of the samples of peas collected from Romney Marsh were affected. An occasional thunder shower and, in a few cases, hail, was

all that most of the crops received throughout the whole period of growth. So dry was the ground that in one crop only about two-thirds of the seed germinated immediately after sowing, the remainder commenced to grow only about three weeks before the more advanced plants were ready for harvest. Only one case was observed in which the seed sprouted in the "wads" after cutting.

It would appear that the hypothetical connection between rainfall and Marsh Spot may lie, not entirely in the direct effect of rainfall upon the plants, but to a great extent in its effect on the water table. It is known that the effect of rainfall upon the water table is not immediate, and the length of time which elapses between the falling of the rain and the response of the water table is dependent on a variety of geological and soil factors. For this reason, when studying the effect of rainfall on the incidence of Marsh Spot, the rainfall of the period immediately preceding the sowing of the peas should be taken into account.

Table IV. *Rainfall*

	Nov.	Dec.	Jan.	Feb.	Sowing		Growing period		Harvest		Sept.
					Mar.	Apr.	May	June	July	Aug.	
1933 season											
Within Romney Marsh:											
Lydd	0.95	0.47	3.03	1.61	1.71	1.19	1.14	1.67	1.29	0.94	5.57
Brookland	1.21	0.32	2.67	1.63	1.69	0.83	1.55	1.08	1.35	0.98	4.44
Dymchurch	0.89	0.32	2.71	1.69	1.65	1.14	1.42	2.19	0.88	0.74	5.31
Outside Romney Marsh:											
Ashford	1.38	0.49	2.27	2.69	2.20	1.55	2.84	2.73	1.47	0.70	3.73
Canterbury	1.30	0.35	1.61	1.23	1.46	0.85	1.24	2.21	1.00	0.66	3.92
Cranbrook	1.36	0.66	2.15	2.20	3.14	1.36	3.67	2.35	2.57	0.94	3.33
1934 season											
Within Romney Marsh:											
Lydd	1.92	0.53	2.62	0.05	3.08	2.13	0.51	1.11	0.95	1.69	0.97
Brookland	1.92	0.40	2.67	0.03	2.55	2.18	0.66	1.36	1.33	2.19	1.06
Dymchurch	1.36	0.45	2.52	0.08	2.46	1.75	0.78	1.14	0.95	1.71	0.93
Outside Romney Marsh:											
Ashford	1.65	0.50	2.58	2.23	3.37	1.87	0.79	1.28	1.75	1.69	0.99
Canterbury	1.17	0.47	2.10	0.23	2.69	2.39	0.87	1.08	0.93	1.61	1.30
Cranbrook	1.44	0.78	3.62	2.26	3.89	2.45	0.72	1.02	0.85	1.87	1.65
South-east England (normal)	3.07	3.11	2.20	2.05	2.05	1.69	1.77	2.17	2.32	2.13	3.44

(d) *Chemistry of the soil*

In the course of the survey 303 samples of soil were collected from pea fields. At each location a sample was taken of the surface soil and one or more from the deeper soil horizons.



Available potash and phosphates were determined by Dr W. Goodwin who used the *Aspergillus niger* method (15). On the basis of previous experience with this method in judging the manurial requirements of arable land, the soils were classified into three groups: adequate, doubtful and deficient. Soils which had produced crops heavily affected with Marsh Spot, however, fell indiscriminately into each category. The conclusion was therefore reached that Marsh Spot is not caused by deficiency of potash or phosphates.

The percentage of lime in each sample was determined, and Mr N. H. Pizer tested the hydrogen-ion concentration by means of the quinhydrone electrode method. Lime was present in abundance in all the soils of Romney Marsh and in the soil on which the plots were laid out at Wye. It was also present in the soil of the *Sharbrooks series* in the field at Brabourne, which was the only case showing any appreciable amount of Marsh Spot, apart from Romney Marsh and the plots at Wye. It is therefore evident that all the heavily affected crops were grown upon soils rich in lime, but the fact that a number of highly calcareous soils produced crops completely free from Marsh Spot suggests that the presence of lime is probably not the primary factor. It seems probable that it was purely accidental that all save one of the soils possessing a water table close to the surface were calcareous.

#### SUMMARY

1. An account is given of Marsh Spot in the seeds of the green pea (*Pisum sativum* L.); its economic importance and its effect on the plant, including the germination of the seed, are discussed.

2. Hypotheses advanced by seedsmen and growers to account for the presence of Marsh Spot are discussed.

3. The soils of 165 fields upon which green peas were grown under contract for seed in east Kent during 1933 and 1934, were surveyed in detail and are classified under the American system. A brief description of each is given.

4. The occurrence of Marsh Spot is correlated with the presence of a water table in the soil. A high percentage of Marsh Spot was encountered only in crops grown on fields with a water table within 52 in. of the surface. Thus, in the presence of a water table, the intensity of Marsh Spot reached a maximum of 100 per cent. of the seeds of a sample affected, whereas in the absence of a water table the maximum was only 3 per cent.

5. On the fields surveyed in Romney Marsh the effect of the water

table was modified by the soil texture. Thus soils with a light texture carried crops with the least average incidence of Marsh Spot, while those with a heavy texture carried crops with the greatest average incidence. The effect of texture upon water movement in the soil is probably responsible. This finding is not without exceptions, and two notable examples are discussed.

6. High percentages of Marsh Spot recorded during two very dry seasons preclude wet weather as an essential primary cause of the disease.

7. Various suggestions as to the cause of Marsh Spot which have, from time to time, been advanced are critically examined. Five of these, viz. a protracted growing period, contact of pods with the ground, the presence of common salt (NaCl) in the soil, deficiency of available potash and phosphates, and lastly the system of cropping, are shown to be totally unconnected with the occurrence of Marsh Spot. The presence of lime in the soils which produced highly affected crops is regarded as coincidental.

8. Manuring with potash, tested as a preventive of Marsh Spot, is shown to have been useless in a very dry season.

The writers offer their warmest thanks to the firms of seedsmen and the numerous farmers whose willing co-operation made the investigation possible.

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# OBSERVATIONS ON THE MINERAL METABOLISM OF PULLETS. II<sup>1</sup>

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(With Four Graphs)

THE phosphorus excretion of pullets increases about the time of shell formation when the diet is not particularly rich in calcium(1, 2, 3, 4). Such temporary increases in phosphorus excretion may be associated with the appearance of water-soluble  $P_2O_5$  in the droppings, although only traces of water-soluble  $P_2O_5$  will be present at other times on the same rations(4). It seemed probable that this water-soluble  $P_2O_5$  represented urinary phosphate, excretion of phosphate having been partly deflected from the normal intestinal route(5) to the urinary route in order to meet a temporary acidotic condition evoked by demands for calcium for shell formation. Experiment showed that the heavy phosphorus excretion did not necessarily involve any increase in the excretion of fixed base, and it therefore appeared possible that the extra phosphorus might be excreted as ammonium phosphate(4).

The present paper deals with a metabolism experiment which was primarily concerned with the investigation of ammonia excretion during the time of shell formation and extra phosphorus excretion. At the same time the experiment was designed so as to provide information on the calcium-phosphorus metabolism of the laying pullet when the calcium carbonate intake is high.

## SCHEME OF EXPERIMENT

The experimental work comprised the determination of the daily intake in the food and output in the droppings of total nitrogen, calcium and phosphorus for four White Wyandotte pullets of the same strain and hatching. The daily output in the droppings of ammonia nitrogen was also determined. The preformed ammonia content of the food was negligible.

The birds were placed in metabolism cages when they appeared to

<sup>1</sup> Abridged form of part of a thesis approved for the degree of Doctor of Philosophy of the University of London.

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be nearing the beginning of laying (July 3, 1934), and were gradually introduced to the experimental rations. The experimental period began on July 11, 1934, but the first four samples of droppings were later discarded.

The experimental rations were carefully mixed by hand and prepared in pellet form by courtesy of a local manufacturer. The compositions of the rations are set out in Table I.

Table I. *Composition of the rations*

	Ration C (high CaO content) parts by weight	Ration NC (low CaO content) parts by weight
Bran	20	20
Pollards	40	40
Sussex ground oats	10	10
Fish meal	5	5
Extracted soya-bean meal	10	10
Yellow maize meal	51	56
Whole wheat meal	51	56
Cod-liver oil	2	2
Sodium chloride	1	1
Calcium carbonate	10	—

Pullets C 1 and C 2 received ration C; pullets NC 3 and NC 4 received ration NC.

### EXPERIMENTAL TECHNIQUE

Three modifications were introduced into the experimental technique described previously (3, 4):

(a) The birds were not irradiated with ultra-violet light, but cod-liver oil was included in the rations.

(b) An attempt was made to regulate the daily food consumption so as to secure a uniform intake from day to day. This attempt was not very successful, for appetite sometimes fell very unexpectedly, but it had the merit of greatly reducing day to day variations in food intake. The birds were fed daily at 9.30 a.m. and again at 4.15 p.m. The droppings for the previous 24 hours were collected daily at 9 a.m.

(c) Analysis of the fresh droppings was impracticable, and hence special treatment was necessary in order to prevent losses of nitrogen during drying. A satisfactory technique has been described by St John and Johnson (6); the fresh excreta are thoroughly mixed with sufficient hydrochloric acid to bring the mass to a pH of 4.0, and the mixture is then dried in the air oven at 50–60° C. The dried excreta may be dried further at 100° C., but this was not always necessary. The amounts of acid necessary per gram of fresh droppings were determined by making

rough titration curves at the beginning of the experiment, and frequent checks were made during the experimental period by means of the quinhydrone electrode. Rectangular Pyrex pie dishes,  $9\frac{1}{2}$  by 6 in., are very suitable for collecting, treating and drying the droppings by this method.

#### ANALYTICAL METHODS

Calcium was determined volumetrically after precipitation as calcium oxalate.

Nitrogen was determined by the ordinary macro-Kjeldahl method.

Phosphorus was determined as follows: A sample containing about 10 mg. phosphorus was weighed into a silica basin and treated with 5 ml. 20 per cent. calcium acetate solution. After drying thoroughly, the sample was ashed, dissolved in 10 ml. 2*N* hydrochloric acid, and made up to 250 ml. Phosphorus was determined on an aliquot of this solution (usually about 10 ml.) by the method of Fiske and Subbarow (7). Using these quantities no correction is necessary for the small amounts of acid present in the aliquot and molybdate I can be used straight away for both aliquot and standard.

Ammonia nitrogen was determined by the following adaptation of the micro-method of Conway and Byrne (8): A sample containing about 0.7–1.2 mg. ammonia nitrogen was weighed into the outer chamber of a standard Conway absorption unit, and 1 ml. *N*/10 sulphuric acid was delivered into the absorption chamber. The sample was then wetted thoroughly with 1 ml. 30 per cent. alcohol, and 1 ml. saturated potassium carbonate solution introduced in the usual manner. Absorption of preformed ammonia was taken as complete after 7 hours at room temperature; there was a small continuous and uniform evolution of ammonia equivalent to about 0.010–0.015 per cent. of the sample per hour after 7 hours, but this was probably derived from sources other than preformed ammonia. For the purposes of the present paper the ammonia nitrogen liberated in 7 hours in the Conway unit under the specified conditions was taken as a close approximation to the preformed ammonia nitrogen present.

Eggs were preserved for analysis by painting with collodion. The shells were removed from the eggs plus membranes and analysed separately. The eggs plus membranes were beaten to a cream with 2 gm. A.R. magnesia, dried, ashed, dissolved in hydrochloric acid, and aliquots taken for the determination of calcium and phosphorus. The phosphorus determinations were made by the method of Richards and Godden (9).

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Calcium was determined volumetrically; several reprecipitations in presence of excess ammonium oxalate and ammonium chloride were necessary on account of the large excess of magnesium.

The analytical data for the rations are given below in Table II. It will be noted from the standard deviations of the averages that sampling and analytical errors were reasonably small.

Table II. *Analytical data for the rations*

	Ration C			Ration NC		
	No. of determinations	Average percentage	s.d. of average	No. of determinations	Average percentage	s.d. of average
CaO	12	3.100	0.0099	12	0.365	0.0031
P <sub>2</sub> O <sub>5</sub>	12	0.930	0.0052	12	0.979	0.0067
N	6	1.939	0.0101	6	2.027	0.0069

A large number of the calcium, phosphorus and nitrogen determinations on the droppings, and all the ammonia determinations, were duplicated. Duplicates agreed satisfactorily.

### EXPERIMENTAL RESULTS

The experimental data are too extensive for complete tabulation, and they are accordingly set out diagrammatically in Graphs I-IV. The unshaded columns represent the daily intake of the constituent in question and the adjoining shaded columns the corresponding output in the droppings. Output in eggs does not appear on the graphs, but the dark circles indicate the days on which eggs were laid; the unshaded circles denote soft-shelled eggs. The data for the eggs are shown separately in Table IV (*vide infra*).

The pullets were weighed about every fortnight before their morning feed. The live weights are given in Table III; it will be seen that Nos. C 1, NC 3 and NC 4 increased in weight during the experiment, while No. C 2 increased at first but then lost weight again during laying.

Table III. *Live weights of the experimental pullets*

Day of exp.	Live weight in kg.			
	Pullet C 1	Pullet C 2	Pullet NC 3	Pullet NC 4
1	1.73	1.51	1.31	1.52
15	1.83	1.66	1.45	1.67
30	1.83	1.89	1.61	1.82
44	1.96	2.01	1.77	2.04
58	1.95	1.72	1.72	1.84
70	1.92	1.66	—	1.98

In the discussion which follows, the "retention" of a constituent means the difference between its intake in the food and its output in the droppings. "Net retention" signifies the intake in the food less the output in droppings and eggs. (The amounts of calcium and phosphorus in the feathers are negligible, and in the present experiment very few feathers were dropped during the experimental period.)

*Pullet C 1 (see Graph I)*

Pullet C 1 behaved satisfactorily, and her food consumption was comparatively high throughout the experimental period. From day 19 until day 24, and again from day 44 until day 50, food consumption fell off and the droppings were loose, but these changes had surprisingly little effect on the retention figures.

Calcium retention was high before laying, and was still higher during laying. The percentage retention, of course, never reached the high values obtained with birds on low calcium rations although the absolute retention figures were very high, and calcium excretion never attained the low level recorded during laying in experiments with low calcium rations. This state of affairs is doubtless due to the fact that the daily intake was usually high enough to supply the calcium for an egg shell.

There were no sudden large alterations in phosphorus excretion in association with laying; this strongly supports the view that, where the calcium carbonate intake is sufficiently high, calcium is transferred directly from gut to oviduct *via* the blood (10).

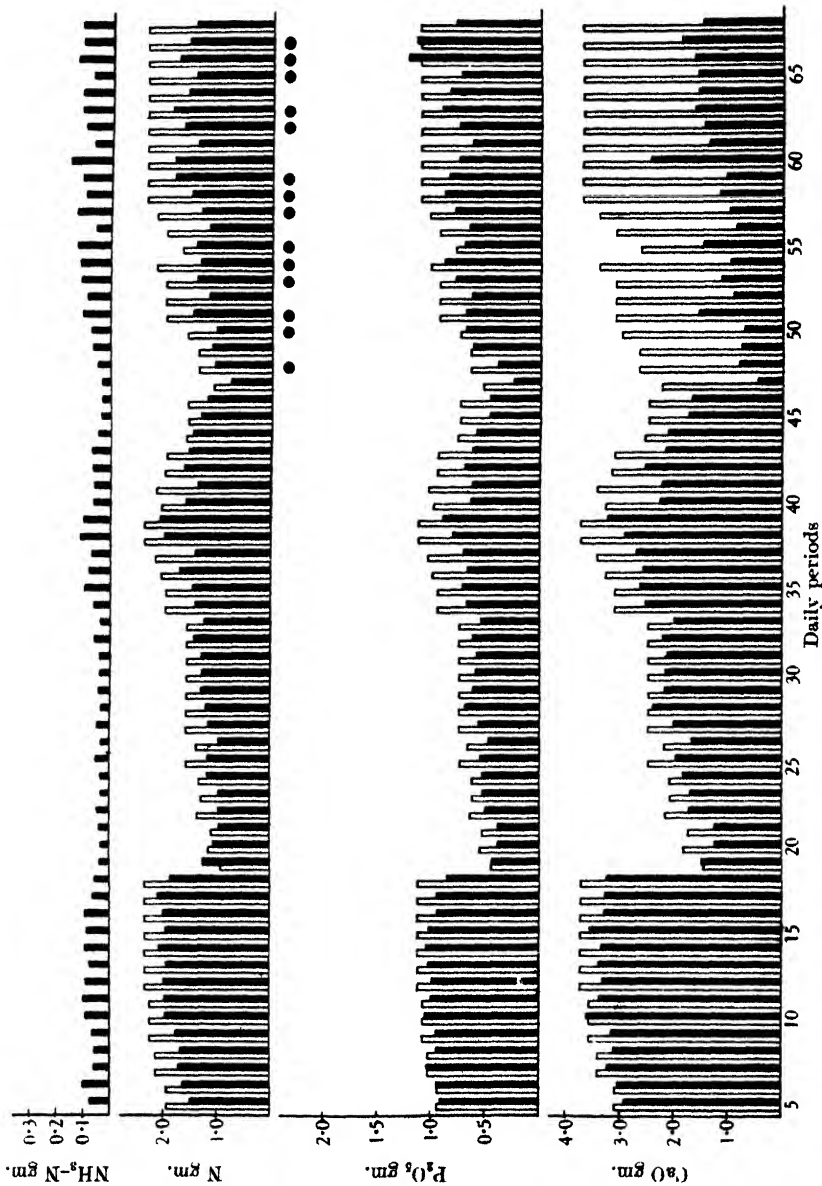
Ammonia excretion showed a tendency to increase during laying in spite of the high calcium carbonate intake. This suggests that there may have been periods of transient acidosis even in the case of pullet C 1 during shell secretion.

Pullet C 1 laid fourteen eggs during the experimental period; and these were all satisfactory pullet eggs. During the laying period 39.53 gm. CaO were retained from the food and 35.86 gm. CaO voided in eggs, leaving a net retention of 3.67 gm. CaO during the laying period.

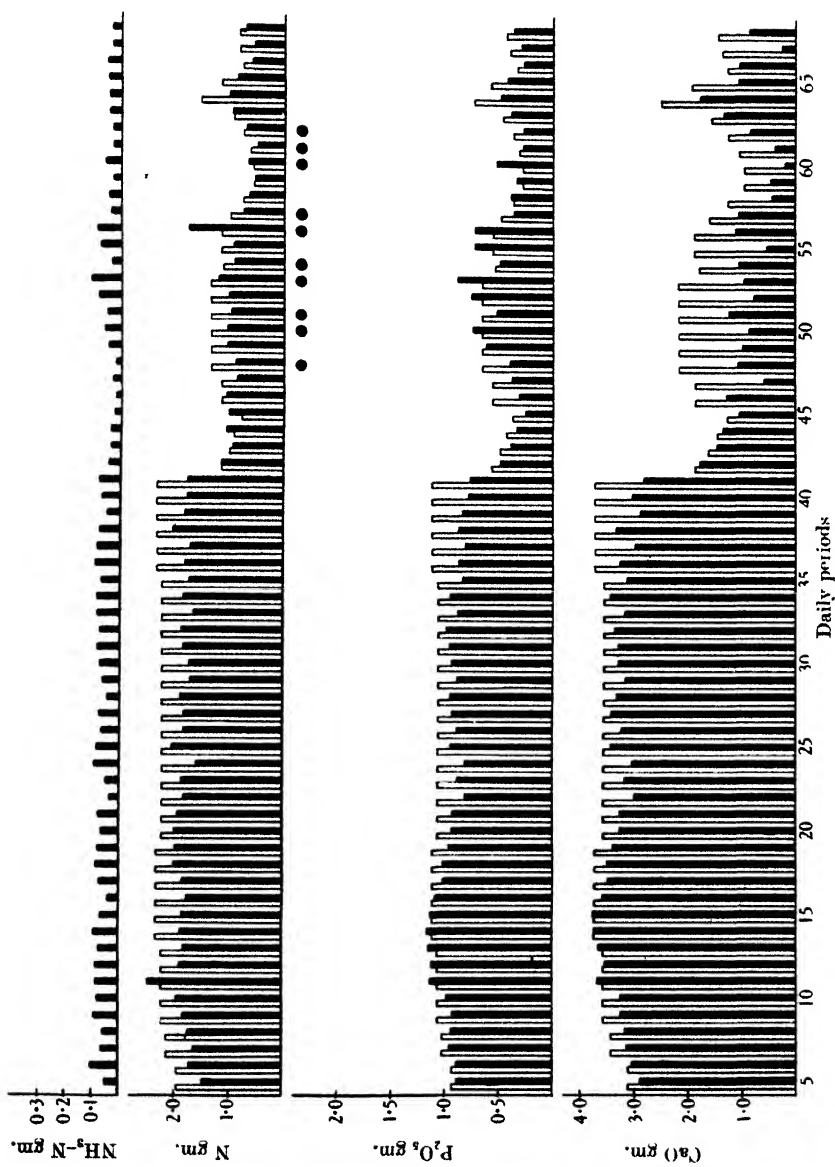
*Pullet C 2 (see Graph II)*

A comparison of the data for pullet C 2 with the data for pullet C 1 proves to be instructive. Food consumption, and hence calcium intake, were much lower in the case of pullet C 2 during the laying period; hence pullet C 2 did not usually ingest sufficient calcium during a daily period to provide for an egg shell. During the entire laying period she retained 13.32 gm. CaO from the food and voided 18.40 gm. CaO in eggs, leaving





Graph I. *Pullet C1.*

Graph II. *Pull C 2.*

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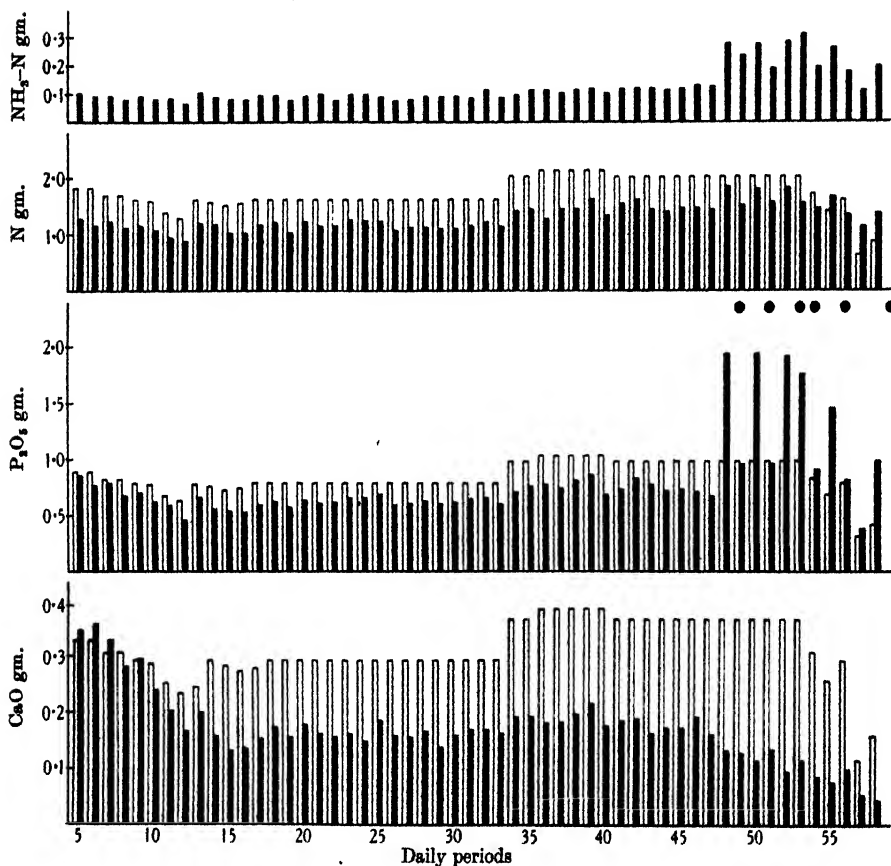
a net loss of 5.08 gm. CaO. During the periods of shell formation pullet C2 displayed a fairly heavy phosphorus excretion, doubtless because of her lower calcium carbonate intake as compared with pullet C 1 (*vide infra*).

Pullet C 2 laid nine eggs during the experimental period. These were lighter than those laid by pullet C 1 and also had lighter shells. On day 57 a curious mass of albumen containing beads and streaks of calcium carbonate was voided; the succeeding eggs appeared to be quite normal except for their somewhat light shells.

### *Pullet NC 3 (see Graph III)*

This pullet was a somewhat unsatisfactory subject for two reasons:

(a) She proved to be infected with roundworms. These were removed by use of a vermifuge on day 43.



Graph III. *Pullet NC 3.*

(b) About day 56 she began moping, blood began to appear in the droppings, and on day 58 she went off her legs. On that day she was killed. Autopsy revealed a large ovarian haematoma which was pressing on the sciatic nerves and thus inducing paralysis.

However, in spite of all this, the pullet was in good condition until day 55, and the data have not been discarded because they agree very well with the data secured from pullet NC 4.

Calcium retention followed a similar course to that recorded in other experiments with birds on low calcium rations. Retention increased gradually before laying began, and during laying the total calcium excretion tended towards the usual low level of about 0.25 gm. CaO per diem.

The periods of shell formation were accompanied by heavy phosphorus excretion.

Even during the prelaying period, ammonia excretion was greater in this bird than in the case of either C 1 or C 2. During the laying period definite and considerable increases in ammonia excretion accompanied heavy excretion of phosphorus.

The nitrogen losses towards the end of the experimental period were attributed in part to bleeding.

Pullet NC 3 laid six eggs during the experimental period. The first three eggs had normal shells, but the succeeding eggs had progressively thinner shells. Pullet NC 3 retained 2.07 gm. CaO from the food during the laying period and voided 10.41 gm. in eggs, leaving a net loss of 7.34 gm. CaO.

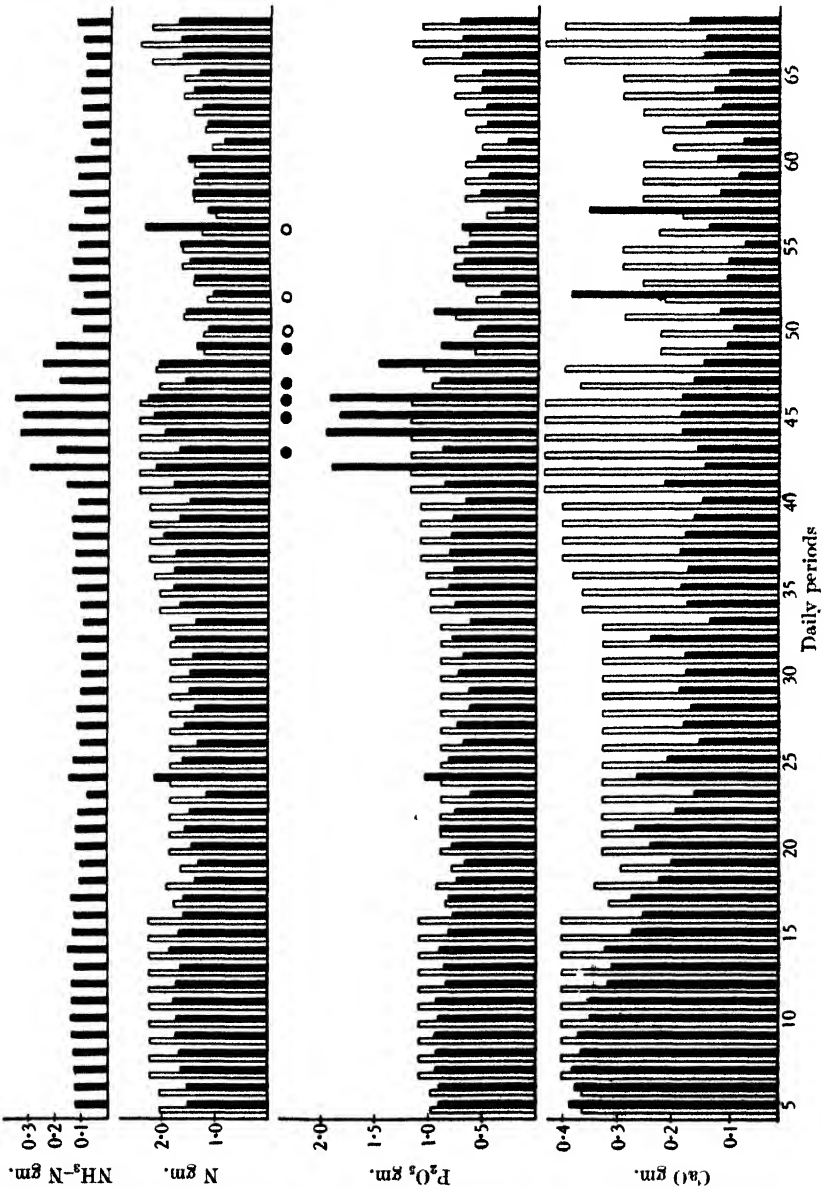
#### *Pullet NC 4 (see Graph IV)*

This bird was an excellent experimental subject, except that the last two eggs which she laid were broken and their shells devoured. This accounts for the heavy voiding of lime on days 53 and 57. It was impossible to separate the egg laid on day 56 from the droppings; the data for constituents voided on day 56 therefore include the constituents of the egg laid on that day. This explains the extra gramme or so of nitrogen in the droppings for day 56.

Calcium retention followed the usual course. There was a gradual increase in retention until the beginning of laying, high retention during the laying period, and lower retention after laying had ceased.

Heavy phosphorus excretion occurred in association with shell formation, and was accompanied in every instance by a large increase in ammonia excretion. This confirms the results secured with pullet NC 3.

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Graph IV. *Pullet NC 4.*

Nitrogen retention was low during the laying period, probably because the intake was low.

Pullet NC 4 laid eight eggs during the experimental period, the six which were recovered being of normal weight for pullets' eggs. The shell of the last egg secured was quite papery.

Table IV. *Composition of eggs laid during the experiment*

Pullet No.	Egg No.	Day of exp.	Total fresh weight	Shell		White + yolk + membranes				Whole egg	
				CaO gm.	P <sub>2</sub> O <sub>5</sub> gm.	CaO		P <sub>2</sub> O <sub>5</sub>		CaO gm.	P <sub>2</sub> O <sub>5</sub> gm.
						gm.	%	gm.	%		
C 1	1	48	41.0	1.958	0.016	0.055	0.15	0.160	0.44	2.013	0.176
	2	50	46.2	2.128	0.015	0.067	0.16	0.187	0.44	2.195	0.202
	3	51	46.7	2.365	0.017	0.070	0.17	0.190	0.45	2.435	0.207
	4	53	49.8	2.480	0.015	0.065	0.14	0.199	0.44	2.545	0.214
	5	54	49.8	2.480	0.016	0.062	0.14	0.209	0.46	2.542	0.225
	6	55	51.2	2.686	0.018	0.063	0.14	0.202	0.44	2.749	0.220
	7	57	52.3	2.656	0.017	0.067	0.14	0.205	0.43	2.723	0.222
	8	58	52.6	2.636	0.016	0.070	0.15	0.205	0.43	2.706	0.221
	9	59	51.3	2.622	0.017	0.064	0.14	0.195	0.42	2.686	0.212
	10	62	47.8	2.446	0.016	0.063	0.15	0.175	0.41	2.509	0.191
	11	63	46.8	2.611	0.018	0.062	0.15	0.173	0.41	2.673	0.191
	12	65	49.7	2.646	0.018	0.075	0.17	0.188	0.42	2.721	0.206
	13	66	51.4	2.590	0.021	0.065	0.14	0.192	0.41	2.655	0.213
	14	67	50.4	2.635	0.020	0.073	0.16	0.186	0.41	2.708	0.206
C 2	1	48	44.0	1.948	0.021	0.057*	0.14	0.174	0.43	2.005	0.195
	2	50	46.5	1.916	0.014	0.061*	0.14	0.192	0.45	1.977	0.206
	3	51	47.8	2.107	0.017	0.056	0.13	0.188	0.43	2.163	0.205
	4	53	47.3	2.029	0.017	0.059	0.14	0.190	0.44	2.088	0.207
	5	54	47.1	2.069	0.020	0.067	0.16	0.208	0.48	2.136	0.228
	6	56	48.9	2.004	0.018	0.067	0.15	0.211	0.47	2.071	0.229
	7	57	—	—	—	—	—	—	—	—	—
	8	58†	—	—	—	—	—	—	—	0.367	0.030
	9	60	44.5	1.821	0.017	0.074‡	0.18	0.174	0.42	1.895	0.191
	10	61	42.7	1.793	0.017	0.067	0.17	0.153	0.39	1.870	0.170
NC 3	1	49	46.4	2.302	0.020	0.064	0.15	0.146	0.35	2.366	0.166
	2	51	47.9	2.209	0.018	0.067	0.15	0.145	0.33	2.276	0.163
	3	53	49.7	2.074	0.017	0.073	0.16	0.172	0.38	2.147	0.189
	4	54	47.8	1.737	0.015	0.084	0.19	0.158	0.36	1.821	0.173
	5	56	47.4	1.718	0.014	0.087	0.20	0.168	0.38	1.805	0.182
	6	59	43.7	1.680	0.013	0.078	0.19	0.164	0.41	1.758	0.177
NC 4	1	43	46.0	2.039	0.014	0.055	0.13	0.164	0.36	2.094	0.178
	2	45	48.7	2.046	0.012	0.106‡	—	0.187	0.38	2.152	0.199
	3	46	49.0	1.590	0.009	0.100‡	—	0.160	0.33	1.690	0.169
	4	47	49.5	1.569	0.010	0.116‡	—	0.140	0.28	1.685	0.150
	5	49	51.0	1.344	0.008	0.110‡	—	0.202	0.40	1.354	0.210
	6	50	—	0.714	—	0.109	—	—	—	0.823	0.214
	7	52	Broken, shell eaten				—	—	—	0.115	0.170
	8	56	Broken, mixed with droppings				—	—	—	—	—

\* Calculated from average for four succeeding eggs.

† Not an egg, but a mass of albumen and CaCO<sub>3</sub> and membranes.

‡ Small fragments of shell present.

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### *Composition of the eggs*

The composition of the eggs laid by the experimental pullets is dealt with somewhat fully in Table IV. Shell weight was calculated from the total calcium content of the shells, assuming that 94 per cent. of the shell is  $\text{CaCO}_3$ . The fresh weight of white plus yolk plus membranes was then obtained by difference, and the percentages of  $\text{CaO}$  and  $\text{P}_2\text{O}_5$  calculated on these figures.

It may be seen from Table IV that the omission of calcium carbonate from the ration resulted in a lower percentage of  $\text{P}_2\text{O}_5$  in the eggs. Most of the phosphorus in the egg is present in the yolk, and it is not possible from the data to say whether the differences in composition were due to (a) differences in the proportion of yolk to white or (b) differences in yolk composition.

### DISCUSSION OF RESULTS

It is evident from the results that heavy phosphorus excretion at the time of shell formation may involve extra ammonia excretion. Previous experiments(4) demonstrated that extra excretion of fixed base is not necessarily involved with this heavy phosphorus excretion. The results are put forward in support of the view that extra phosphorus excretion at the time of shell formation takes place to some extent at least in the form of ammonium phosphate *via* the urinary route. Base economy is presumably effected by ammonia excretion rather than by excretion of fixed base; some evidence has been secured in this laboratory that base economy in the pullet during extra phosphorus excretion may also be effected partially by decreased chlorine excretion(11). It remains to be seen whether the fowl effects base economy under these circumstances by increasing ammonia excretion at the expense of uric acid excretion.

The data for pullet C 1 confirm the view that, when the calcium carbonate intake is sufficiently high, lime is transferred directly from the gut to the oviduct *via* the blood stream(10, 12). No extra phosphorus excretion is involved under these conditions. When the calcium carbonate intake is lower, and insufficient passes into the intestinal tract during the period of shell formation to provide calcium for an egg shell (see Graph II, pullet C 2), a certain amount of extra phosphorus excretion occurs. Extra phosphorus excretion does not necessarily of itself indicate an attack on body reserves of calcium phosphate for calcium. As pointed out by McGowan(22), during shell secretion there is a heavy demand for calcium, and if practically all the calcium ingested is being deflected to the oviduct, then no appreciable amounts of phosphate will be held by

the tissues, and phosphate which would otherwise be fixed as some form of calcium phosphate will be excreted. This source of increased  $P_2O_5$  excretion cannot, however, account for a total  $P_2O_5$  excretion considerably in excess of the total  $P_2O_5$  intake during the period of shell formation. Where  $P_2O_5$  excretion is greatly in excess of the corresponding intake, clearly the excess must originate in large part from bodily reserves, *i.e.* presumably from skeletal reserves of calcium phosphate which are being depleted for shell formation. Obviously, individual cases must be considered on their merits from a quantitative standpoint.

It would appear that calcium and phosphorus metabolism during shell formation are subordinated to the necessity for the rapid mobilisation and secretion of large amounts of calcium in the shell. There is a certain amount of evidence that the parathyroid glands are concerned in the calcium metabolism of shell production (13, 14, 15); the exact part they play is still obscure, and it seems that the hypercalcaemia of the laying hen is in large part of a different nature to parathyroid hypercalcaemia (16). The response of the fowl to parathyroid hormone, however, is rapid and sudden (15); certainly parathyroid activity would provide an efficient mechanism for a rapid call on skeletal reserves and aid in sweeping out excess phosphate from the circulation (17).

The data from the present experiment provide evidence that skeletal reserves were drawn upon in order to provide calcium for shells in the cases of pullets NC 3 and NC 4. The relevant figures are set out in Table V.

Table V

Pullet	CaO retained during laying period gm.	CaO lost in eggs gm.	CaO lost by body during laying period gm.	CaO stored during 3 weeks before laying gm.
C 1	39.53	35.86	(Gained 3.67 gm.)	12.50
C 2	13.32	18.40	5.08	8.94
NC 3	2.07	10.41	7.34	3.57
NC 4	2.07	9.91	7.42	3.57

In the case of pullets NC 3 and NC 4 the prelaying storage of calcium was insufficient to make up for the losses during the laying period.

The "tibia ash" and serum phosphatase figures for the pullets at the end of the experiment also suggest interference with skeletal metabolism in the case of pullet NC 4. The data are presented in Table VI. (Pullet NC 3 was killed earlier for autopsy, but a subsequent *ad hoc* experiment with four similar birds receiving the same rations supports the validity of the figures given in Table VI.)

The  $CaO/P_2O_5$  retention ratios secured with pullets C 1 and C 2 were



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exceptionally high, and suggested storage of calcium in forms other than tricalcic phosphate. The ratios for these birds were the highest which have been noted in this laboratory (4); the  $\text{CaO}/\text{P}_2\text{O}_5$  ratio of the food was also higher than that used in other experiments. The data for the present experiment are set out in Table VII.

Table VI

Pullet	Tibia ash (dry, fat-free, basis)	Serum phosphatase (Bodansky units)
	%	
C 1	61.8	17.4
C 2	61.0	6.4
NC 4	55.2	184.0

Table VII

Pullet	CaO retention (3 weeks before laying)	$\text{P}_2\text{O}_5$ retention (3 weeks before laying)	$\text{CaO}/\text{P}_2\text{O}_5$ retention ratio	$\text{CaO}/\text{P}_2\text{O}_5$ ratio of food
	gm.	gm.		
C 1	12.50	5.03	2.48	3.33
C 2	8.94	3.78	2.36	3.33
NC 3	3.57	4.44	0.80	0.38
NC 4	3.57	4.08	0.91	0.38

### *Nitrogen metabolism*

Nitrogen retention varied considerably from day to day, although daily nitrogen intake was often uniform for days at a time. Willcox (19) found that nitrogen retention in laying hens varied considerably from day to day, but that retention was related to nitrogen intake; the relation is slightly accentuated by his method of graphical presentation, and his tables of data show that the variations which he encountered were not unlike those met with in the present experiment. Willcox points out that it is difficult to see how a valid daily balance can be struck between egg nitrogen and food nitrogen owing to the time required for egg formation. He was able to secure an average daily balance which was probably very close to the truth by using hens in full lay and keeping them on balance for 10 weeks. In the present experiments the laying periods were short, and the birds fluctuated somewhat in live weight. As a result Willcox's method of securing a figure for the digestible nitrogen requirement for egg production is not applicable to the data of the present experiment, except to a limited extent in the case of pullet C 1. The fourteen eggs laid by this bird from day 48 until day 67 may be balanced against the nitrogen metabolism from day 47 until day 66 in order to secure an approximate figure for the amount of digestible nitrogen required to produce a unit of egg nitrogen.

For the period in question the nitrogen intake was 39.96 gm., and the nitrogen voided in the excreta 27.79 gm. The total weight of the eggs less shells was 619.6 gm.; this is equivalent to 12.77 gm. egg nitrogen, for the total nitrogen content of fresh eggs less shells is about 2.06 per cent. (18). The net nitrogen balance was therefore  $-2.10$  gm., and this may be assumed to represent digestible nitrogen derived from the body and completely utilised for egg production. The digestibility of the nitrogen in the ration was calculated from the available data on digestibility (20, 21) and estimated at 80.2 per cent. The weight of the bird was 1.94 kg.

By applying the arguments of Willcox to these data, a figure of 2.24 gm. digestible nitrogen is secured as the requirement for the production of 1 gm. egg nitrogen. This figure is of the same order as those secured by Willcox, and supports his contention that current tentative feeding standards pitch the digestible protein requirements for egg production at too high a level.

This reasoning was not applied to the remaining pullets on account of the small numbers of eggs laid, and the manifest disturbance of egg production in the case of the pullets receiving the low calcium ration.

#### SUMMARY

1. Where heavy phosphorus excretion accompanies egg laying in the pullet the excretion of ammonia nitrogen is simultaneously increased. It is probable that this indicates an excretion of excess phosphate in the urine as ammonium phosphate.

2. It is shown that heavy phosphorus excretion does not accompany egg laying provided the calcium carbonate intake is sufficiently high.

3. The origin of the excess of phosphorus excretion is discussed in relation to calcium-phosphorus metabolism.

4. Pullets on a ration containing 5 per cent. calcium carbonate laid eggs containing a higher percentage of  $P_2O_5$  than pullets receiving a similar ration but from which the calcium carbonate supplement was omitted.

5. Some evidence is put forward in support of the view that current standards pitch the requirements of digestible protein for egg production at too high a level.

The author wishes to thank his colleagues for their interest and encouragement, and J. Johnston for his careful management of the experimental birds.

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# THE RELATION OF PRODUCTION TO MORTALITY IN THE DOMESTIC FOWL

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MORTALITY in the laying flock has increased to alarming proportions during recent years. This increased mortality is general throughout poultry flocks, on the American continent at least, as the published records of various investigators show. During the course of a general study on the inheritance of egg production, an apparent relation between the rate of laying and the incidence of mortality among birds entered in Canadian Egg-Laying Contests appeared to provide a disturbing influence.

Harris<sup>(1)</sup> published data from the Storrs Egg-Laying Contest from which he concluded that birds which died at some time during their first laying year were lower producers, previous to death, than birds which survived throughout the year. White Leghorns supplied the data upon which this conclusion was based. The same author<sup>(2)</sup>, using data from the same source, extended his observations to Rhode Island Reds and White Wyandottes, drawing the same conclusions in respect to these breeds.

There are, however, certain conditions which must be borne in mind in connection with the data of Harris which leave his conclusions not entirely beyond criticism. The question under investigation is whether there is any direct relationship between the laying ability of the individual female and her chances of survival. The laying contest at Storrs is composed of more or less independent units or pens, each of which contain a number (thirteen in the laying year 1934-5) of females. It is a well-known fact that differences exist in the laying ability as well as the death-rate of these pens. These differences arise from one or more causes of variation, many of which are quite independent, on account of the fact that the pens originate from private breeders in widely separated parts of the country. Among others, these independent causes of variation include differences in genetic constitution, as well as differences in feeding methods, housing conditions and exposure to disease during the rearing period. These differences undoubtedly influence not only the egg

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production but also the mortality rate of the individual pens. In addition differences exist between years at the same contest which undoubtedly influence both production and death-rate. These include variations from year to year in climatic conditions, feeding methods and particularly the incidence of infectious diseases.

For these reasons it is obvious that when the individual birds in the Storrs Contest for a period of ten years are lumped together in one population, as Harris has done, that population does not fulfil the conditions of simple sampling. A valid comparison of the laying ability of birds which die and those which survive cannot be made unless differences due to pens and to years do not contribute to the differences between those dying and those surviving. In view of the relatively large and consistent difference secured by Harris, however, one would expect to find an actual difference in favour of survivors even when differences due to years and pens were removed.

Hays and Sanborn(3), contrary to the findings of Harris, conclude that, in their strain of Rhode Island Reds, the winter production of birds dying after March 1 is no less than that of birds surviving throughout the year. In this case also data of five years are lumped together in the one population. The difference between the means of the dying and surviving birds is 1.72 in favour of survivors with a probable error of 3.38. Examination of the data presented in Table II of their report reveals that, in three of the five years, the surviving birds exceeded those dying, in one of the remaining two the difference in favour of those dying is only 0.01 egg, while in 1926 the difference in favour of the survivors is 6.51 with a probable error of 2.02 and is, therefore, statistically significant. When the means for the two groups for each of the five years are regarded as independent paired observations, the mean difference in favour of the survivors is 2.62 with a standard error of 1.208. This gives a value of 2.169, the 0.05 point in Fisher's table of *t* being 2.776. While the pooled data of Hays and Sanborn do not permit one to draw definite conclusions concerning the superiority of survivors, there are indications that this is so particularly when the results of 1926 are considered.

These appear to be the only published reports dealing with the relation of the individual's laying ability to the mortality rate, although Weaver and Bird(5) show that birds which were autopsied as "cannibalised" among cadavers from the Ottawa Experimental Farm flock were poorer layers than those surviving throughout the year. In presenting mortality figures on a population of 4000 White Leghorns and 1810

Barred Plymouth Rocks collected over a period of five years, Weaver(4) finds a seasonal relationship between the rate of production and mortality, in which as production rises there is a tendency for mortality to rise. This appears to be a generally accepted idea as evidenced by the following statement of Hays and Sanborn(3): "In pedigree breeding for high fecundity, the question of vigor is of paramount importance, because the mortality rate tends to become higher as egg production increases. Without question, the complex physiological process of egg laying places the pullet under a severe test for vigor which may or may not shorten her life." It is difficult to reconcile this statement with the findings of Harris. If the strain of heavy egg laying shortens the life of the individual bird, then the heavy layers should show a higher death-rate than the poorer ones. It is quite conceivable, however, that there may be a positive seasonal correlation between the rate of laying of a flock as a whole and the death-rate of the flock due to coincident but causally independent seasonal variation in both of these attributes, whilst at the same time there is, during any given period, a negative correlation between the laying ability of individual birds within the flock and the probability of their death.

With the hope of clarifying the contradictory evidence contained in the literature a study of the exact relation of production to mortality in Canadian Egg-Laying Contests was launched.

The year 1929-30 was chosen as a typical year in so far as mortality was concerned. The average mortality in the Barred Rocks throughout eleven contests during this year was 18.9 per cent., with a range from 14 to 25 per cent. During this year the contests were generally free from severe outbreaks of such infectious diseases as laryngotracheitis and pox. The causes of death are, presumably, typical in nature and incidence to those generally existing in Canadian poultry flocks.

#### RELATION BETWEEN THE LAYING RATE OF THE INDIVIDUAL AND HER CHANCES OF SURVIVAL

The main point in question is whether the heavy laying bird is more or less likely to die than the poorer layer, and consequently whether practices designed to increase the laying ability of the flock, such as breeding and feeding for heavy egg production, are likely also to increase the mortality rate. Extremely careful treatment of the data is necessary in order to answer the question. In addition to the difficulties, previously mentioned, which are inherent in the use of contest data, it is also true that many of the birds which ultimately die pass through a period of

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decline just previous to death, during which they produce no eggs. The lack of egg production during this period, in most cases, is probably not independent of the cause of death, and therefore should not contribute to, and cause a decrease in, the calculated laying ability of such birds. The data in this study were secured from the records of ten pens of each of two breeds, White Leghorns and Barred Rocks, chosen at random from each of nine contests located in each of the nine provinces of Canada with the exception of Alberta. Two of the nine contests were located in Quebec. In cases where ten pens, in which one or more deaths had occurred, were not available for one breed, in any one contest, all pens for that breed in that contest were used. In all a total of 560 Leghorns and 710 Barred Rocks comprised the population.

The contest records are divided for convenience into thirteen 4-week periods throughout the year. Since the contest year extends through only 51 weeks, the thirteenth period is composed of only 3 weeks. The records in only the first twelve periods were used in this study.

For our purpose each pen in each contest was regarded as a unit, and comparisons between the egg production of dying and surviving birds were made within each pen. Furthermore, the egg-laying ability of each bird was calculated as a unit and expressed as percentage production from first egg to last egg, the comparisons taking the form of paired observations within the same time limit. For example, of the ten birds comprising the pen whose records are given in Table I, three died during the year. Of these three, one died during the fourth period, one during the seventh and one during the tenth. Each of these three birds ceased producing for one or more periods previous to death; the period of last egg in each case being the first, sixth and eighth respectively. As seen in column 4, 11 days elapsed between the first and last eggs laid by the bird which died in the fourth period but ceased production in the first. During these 11 days she laid seven eggs, which gives her a production rate of 63.6 per cent. The seven birds which survived throughout the year laid at an average rate of 71.6 per cent. from their first to last eggs during period 1. This gives one direct comparison, viz. 63.6 and 71.6 for dying and surviving birds respectively. Similarly, the bird which died in period 7 laid at the rate of 54.0 per cent. from first to last egg, while the seven birds which survived throughout the year laid at an average rate of 54.1 per cent. from first to last eggs within the same period of time. This gives a second pair of observations of 54.0 and 54.1. Again in the case of the remaining death the paired observations are 53.9 and 60.8.

The data in Table I are those from the White Leghorn pen No. 59 in the Ontario Contest. In Table II the summarised data for the ten White Leghorn pens from this contest are given.

Table I. *Individual production records of the birds which died, as well as those which survived throughout the year for a typical pen in one of the contests, showing the method of computing the paired observations*

Hen No	Died				Survived (periods with eggs over days and rate of laying)											
	Period of last egg	Period of death	Eggs over days	Rate of laying	1	2	3	4	5	6	7	8	9	10	11	12
591	1	4	7 11	63.6	—	—	—	—	—	—	—	—	—	—	—	—
592	—	—	—	—	20 24	83.3	—	—	—	115 165	69.7	156 221	70.6	—	—	—
593	8	10	110 204	53.9	—	—	—	—	—	—	—	—	—	—	—	—
594	—	—	—	—	14 22	63.6	—	—	—	79 162	48.8	108 197	54.8	—	—	—
595	—	—	—	—	18 25	72.0	—	—	—	101 166	60.8	146 222	65.8	—	—	—
596	—	—	—	—	15 23	65.2	—	—	—	89 168	41.1	111 224	49.6	—	—	—
597	—	—	—	—	20 27	74.1	—	—	—	115 167	68.9	168 223	75.3	—	—	—
598	—	—	—	—	10 15	66.7	—	—	—	55 155	35.5	99 213	46.5	—	—	—
599	—	—	—	—	16 21	76.2	—	—	—	87 161	51.9	136 217	62.7	—	—	—
600	6	7	88 163	54.0	—	—	—	—	—	—	—	—	—	—	—	—
Average rate					71.6					54.1			60.8			

In cases where more than one death and consequently more than one paired observation occurs within each pen, a single paired observation for that pen is secured by averaging the paired observations within each pen. This is given in the right-hand column of Table II. In this way one secures, for the White Leghorns in the Ontario Contest, a series of ten paired observations which enables one to calculate the actual difference existing between the rate of production for the dying and the surviving birds, without allowing differences in the general level of production between pens to influence the final result. If the difference between the dying and surviving birds is not constant throughout the twelve periods, *i.e.* if it either increases or decreases as the season advances and if a majority of the paired observations from any one contest are secured either during the first or the last half of the season, then the difference secured by eliminating differences between pens as outlined



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above may be influenced by differences in death rate between periods. This can be corrected by averaging the columns in Table II and thus securing for each contest a series of paired observations which are not influenced by differences in the general production level between periods.

Table II. *Rates of laying for the dying and surviving birds in each pen for the White Leghorns in the Ontario Contest, showing the method of pooling the data from each contest. The final row gives the series of paired observations uninfluenced by differences between pens, while the final column gives the series uninfluenced by differences between periods*

		Periods in which last egg of dead birds produced, with the average rates of laying for those which died and those which survived to and including the period of last egg												Averages by pens	
Pen No.		1	2	3	4	5	6	7	8	9	10	11	12		
31	Died	—	—	—	—	—	10.0	—	—	—	—	—	—	10.0	
	Survived	—	—	—	—	—	48.1	—	—	—	—	—	—	48.1	
33	Died	—	—	—	—	8.4	—	—	—	—	—	—	—	8.4	
	Survived	—	—	—	—	43.9	—	—	—	—	—	—	—	43.9	
34	Died	—	—	—	9.4	—	—	34.3	—	—	—	—	—	21.9	
	Survived	—	—	—	39.6	—	—	45.7	—	—	—	—	—	42.7	
35	Died	—	—	—	—	—	—	53.9	—	—	—	—	—	53.9	
	Survived	—	—	—	—	—	—	70.5	—	—	—	—	—	70.5	
38	Died	—	—	—	21.1	—	—	47.5	—	—	—	—	—	34.3	
	Survived	—	—	—	49.2	—	—	54.4	—	—	—	—	—	51.8	
45	Died	—	—	34.0	—	—	15.1	59.1	32.2	—	54.7	—	—	39.0	
	Survived	—	—	30.1	—	—	23.5	27.4	29.2	—	28.7	—	—	27.8	
48	Died	—	52.0	—	—	—	—	—	49.4	—	—	—	—	51.1	
	Survived	—	59.0	—	—	—	—	—	55.6	—	—	—	—	57.3	
54	Died	—	51.2	—	—	—	—	—	—	—	43.6	—	—	47.4	
	Survived	—	56.1	—	—	—	—	—	—	—	49.1	—	—	52.6	
58	Died	—	—	—	—	—	52.8	34.5	—	—	—	45.7	—	44.3	
	Survived	—	—	—	—	—	46.0	50.3	—	—	—	49.2	—	48.7	
59	Died	63.6	—	—	—	—	—	54.0	—	53.9	—	—	—	57.2	
	Survived	71.6	—	—	—	—	—	54.1	—	60.8	—	—	—	62.2	
Averages by periods:															
	Died	63.6	52.1	34.0	15.3	8.4	33.0	45.9	45.2	—	49.1	45.7	—	—	
	Survived	71.6	57.6	30.1	44.4	43.9	43.1	49.7	48.5	—	38.9	49.2	—	—	

By following this procedure for each breed in each of the nine contests, we secure for each breed in each contest one series of paired observations not influenced by differences between pens and a second calculated from the same data but with differences due to periods removed. Since no White Leghorn pens were entered in the New Brunswick Contest there are sixteen series of paired observations for Leghorns and eighteen series for the Barred Rocks. Each of these thirty-four series may be treated statistically; and the size and significance of the difference in rate of production for each calculated according to "Student's" method for paired observations. Table III shows the mean difference in favour of survivors, together with  $n$ ,  $t$  and  $P$  values for each of the thirty-four series.

Table III. *Showing the mean differences in rate of production in favour of survivors in each of the thirty-four series together with the values for  $n$  and  $t$ , and the necessary value of  $t$  for a  $P$  level of 0.05 and 0.01*

	White Leghorns					Barred Rocks				
	Mean difference	<i>n</i>	<i>t</i>	Necessary <i>t</i> for <i>P</i> of		Mean difference	<i>n</i>	<i>t</i>	Necessary <i>t</i> for <i>P</i> of	
				0.05	0.01				0.05	0.01
Differences due to pens eliminated.										
Prince Edward Island	10.15	1	2.206	12.706	—	4.95	9	2.178	2.262	—
Nova Scotia Southern	9.70	8	1.165	2.306	3.355	11.16	6	1.409	2.147	—
New Brunswick	—	—	—	—	—	6.23	7	2.490	2.365	3.499
Quebec Eastern	4.96	4	1.477	2.776	—	11.79	9	1.842	2.262	—
Quebec Western	17.00	1	2.643	2.776	—	1.23	5	0.180	2.571	—
Ontario	13.81	9	3.047	2.262	3.250	1.88	9	0.448	2.262	—
Manitoba	1.93	7	0.678	2.365	—	5.26	7	1.585	2.365	—
Saskatchewan	12.31	6	7.297	2.447	3.707	0.80	8	0.317	2.306	—
British Columbia	-0.20	9	0.107	2.262	—	6.10	2	0.977	1.303	—
Differences due to periods eliminated.										
Prince Edward Island	7.93	2	2.098	4.303	—	7.81	9	2.501	2.262	3.250
Nova Scotia Southern	10.47	8	1.471	2.306	3.355	15.33	7	2.533	2.365	3.499
New Brunswick	—	—	—	—	—	4.27	8	1.790	2.306	—
Quebec Eastern	1.22	4	0.584	2.776	—	11.19	6	3.065	2.117	3.707
Quebec Western	12.28	5	2.903	2.571	4.032	0.46	6	0.112	2.117	—
Ontario	8.47	9	1.784	2.262	—	5.09	7	1.055	2.365	—
Manitoba	0.41	8	0.128	2.306	—	3.07	6	1.364	2.447	—
Saskatchewan	11.76	9	7.259	2.262	3.250	3.78	8	1.107	2.306	—
British Columbia	-1.94	8	0.599	2.306	—	9.93	2	2.658	4.303	—

With the exception of the British Columbia White Leghorns, the surviving birds in each of the thirty-four series exceeded those dying. In ten of the cases the  $t$  value exceeded the 0.05 point, and in four cases it exceeded the 0.01 point. This table shows a very decided tendency for the birds which survive throughout the year to be heavier layers than those which die.

The size of the difference in production between those dying and those surviving may vary from pen to pen and from period to period. Since all periods or pens are not represented in any row or column (see Table II), the marginal averages will be unequally weighted, and hence the effect of these variations is not entirely eliminated. It is rather difficult to see how the effect of these variations can be overcome. If the data from all contests were combined by individual periods and a  $t$  test performed for each period, the difficulty would be only partially met, since all contests do not contribute data for all periods and since, in many contests, one or very few pens contribute to certain periods. In any case this unequal weighting is randomised through the data, and furthermore, it is clear that it does not materially influence the results.

The data may be pooled by regarding the average rate of production of the birds dying and those surviving in each contest as a final series

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of paired observations and treating it statistically in the same way. In this way differences between contests as well as most of the effects of unequal weighting will be eliminated. There will be two such final series for each of the two breeds, viz. one with differences between pens eliminated and a second with differences between periods eliminated. The results of the pooled data are shown in Table IV.

Table IV. *Mean difference in rate of production in favour of survivors for the pooled data for each breed, together with statistical constants and significance points*

Breed	Differences due to contests and pens eliminated					Differences due to contests and periods eliminated				
	Mean difference	n	t	Necessary t for P of		Mean difference	n	t	Necessary t for P of	
				0.05	0.01				0.05	0.01
White Leghorns	8.71	7	4.406	2.365	3.499	6.325	7	3.419	2.365	3.499
Barred Rocks	5.49	8	4.396	2.306	3.355	6.77	8	4.599	2.306	3.355

With the exception of the Leghorns when differences due to periods are eliminated, the *t* values exceed the 0.01 error point. We are, therefore, quite justified in drawing the conclusion that under conditions such as those existing in Canadian Egg-Laying Contests during the year 1929-30, birds which die at some time during the year are poorer layers during their productive life than pen mates which survive throughout the year. The surviving Leghorns are approximately 7 per cent. better layers than those dying, while the surviving Rocks are approximately 6 per cent. better.

The foregoing analysis shows that the heavier laying birds within a flock are, on the whole, more resistant to the causes of death than the poorer layers. One is not justified in concluding, however, from the above analysis, that all or any of the practices resorted to in efforts to improve egg production will also result in lowering the mortality rate. One flock may exhibit a higher rate of laying as well as a heavier rate of mortality than a second flock, and still in both flocks it may be the poorer layers that succumb. In other words, when a flock as a whole is "forced" for egg production the death-rate may increase, due to heavier mortality among the poorer layers. Although it is a widely held view that high mortality is associated with heavy egg production, it seems just as probable that the egg-producing ability of a flock is a criterion of the health of that flock, and that high-producing flocks are in a better state of health, particularly in regard to the non-communicable diseases, than lower producing flocks. We should, therefore, expect the death-rate

of the higher producing flock to be less than that of the lower flock. On the other hand, if the strain of heavy egg production tends to cause deaths, then, on the average, the death-rate in heavy laying flocks should be higher than in poorer flocks, or, in other words, there should be a positive correlation between production and mortality between flocks. This point can be settled by referring to contest data.

It is a well-known fact that the average egg production of Canadian contest birds is at the present time considerably higher than that which occurred at the inauguration of contests. Likewise the mortality rate is considerably heavier than at the beginning. These increases in production and mortality have occurred as the result of reasonably uniform yearly increments, although closer examination will reveal fairly rapid increases during the first 8 years with a tendency to level off and remain constant, with the exception of chance variations, during recent years. The concomitant rise of production and mortality, however, does not necessarily indicate a correlation between the two. The rise in production is due to improvements in feeding, breeding, management, or a combination of these, while the increase in mortality may be due to a general spread of infective agents and a widespread "seeding" of the country as a whole, and thus entirely independent of the improvements in feeding or breeding. Proper statistical treatment of contest data will reveal whether mortality is dependent or independent of production.

Table V shows the analysis of variance and co-variance, average egg production of surviving birds and rate of mortality for the Barred Rocks entered in all Canadian Egg-Laying Contests during the laying years 1919-20 to 1931-2 inclusive. This table shows clearly that the correlation of 0.368 secured between production and mortality when the figures are treated as a single population is due entirely to the simultaneous rise, which has occurred from year to year, in both production and mortality and is not due to any relationship between the two. This is clearly shown by the high correlation between means of years of 0.787 as compared to the insignificant net correlation of  $-0.078$  between production and mortality.

Although differences due to years are eliminated in Table V, differences between pens within contests might have influenced results. For instance, the majority of deaths may have occurred in the higher producing pens in each contest, but the average production of the poorer birds which survived may have been the equal of surviving birds in contests with lower death-rates.

Table VI shows the pooled data, analysis of variance and co-variance,

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Table V. *Analysis of variance and co-variance, average egg production of surviving birds and percentage mortality. Data from the Barred Rocks in Canadian contests from the year 1919-20 to 1931-2 inclusive*

Co-variance										
Source of variation	Degrees of freedom	Production		Mortality		Sum of products	$r$	Necessary $r$ for $P$ of		Regression of mortality on production
	Sum of squares	Mean square	Sum of squares	Mean square	0.05			0.01		
Total	129	66285.6	513.8	6934	53.8	7894	0.308	0.173	0.226	0.119
Between years	12	37084.6	3090.4	2980	248.3	8270	0.787	0.553	0.684	0.223
Between contests	10	9658.6	965.9	965	96.5	218	0.071	0.602	—	—
Remainder	107	19542.4	182.6	2989	27.9	-594	-0.076	0.188	—	—

Table VI. *Analysis of variance and co-variance, average egg production of surviving birds and number of deaths in the original ten-bird pens of eleven contests. Pooled data from the Barred Rocks for the years 1929-30 and 1930-1*

							Co-variance			
Source of variation	Degrees of freedom	Production		Mortality		Sum of products	$r$	Necessary $r$ for $P$ of		Regression of mortality on production
		Sum of squares	Mean square	Sum of squares	Mean square			0.05	0.01	
Total	178	108761	611	430	2.4	- 1537.4	-0.225	0.147	0.196	-0.014
Between contests	18	35490	1972	81	4.5	- 728.6	-0.430	0.456	—	—
Within contests	160	73270	458	349	2.2	- 808.8	-0.160	0.155	0.208	-0.011

of eleven Canadian Egg-Laying Contests for 2 years 1929-30 and 1931-2, showing the correlation between the average production of surviving birds and the number of deaths in each pen.

This table clearly shows that fewer deaths occur in higher producing pens. The negative correlation between production and mortality is not high, but it should be pointed out that, since it is the lower producing birds which die in each pen, as shown by the previous analysis, the surviving birds are, in a sense, a selected group; this selection is more pronounced in pens suffering the greater losses. The average production of the survivors in pens with high mortality is higher, on account of this process of selection, than the true average for the original ten birds. This would tend to lower the size of the negative correlation, but in spite of this fact the correlation remains sufficiently high to be significant.

If Canadian contests are typical of flocks in general it may be concluded that heavy laying birds are less likely to die than poor layers, and that heavy laying flocks suffer fewer deaths than poorer layers. No evidence has been secured which indicates that heavy egg laying places a strain on the individual which may shorten her life. In fact it appears that the causes of high egg production also exert a favourable influence

on mortality. Adequate feeds, favourable genetic make-up, freedom from disease, both acute and chronic, and proper management all contribute towards the physiological well-being of the animal organism. In the domestic fowl this well-being is reflected in increased egg production and improved health which results in fewer deaths.

The tendency for egg production to be negatively correlated with mortality rate does not preclude the possibility of a positive seasonal correlation between the rate of production and mortality, *i.e.* it is quite possible that, although the deaths within a flock occur in general among the poor layers, the majority of these deaths occur during periods of the year when the rate of production in the flock is higher than average. This is indicated by the work of Weaver(4), who secured seasonal correlations of 0.78 and 0.81 for White Leghorns and Barred Rocks respectively between the rate of production and the death-rate. Here again the correlations have been secured by lumping together 5 years' results of two contests. In addition to the disturbing influence of these differences between contests and between years, differences between periods within the year might exert a pronounced effect upon the size of the ultimate correlation. For example, if differences in the levels of both production and mortality were related to season but actually were independent of each other, then significant correlations might be secured if the effect of these differences between means of seasons was not removed.

Table VII is the analysis of variance and co-variance for twelve periods of the year, showing the correlation between rate of production and rate of death.

Table VII. *Analysis of variance and co-variance, showing the relation between the rate of laying and rate of mortality for twelve periods of the year. Data from the Barred Rocks in nine contests for the year 1929-30. Differences between contests are not included in the total*

							Co-variance		
Source of variation	Degrees of freedom	Production		Mortality		Sum of products	$r$	Necessary $r$ for $P$ of	
		Sum of squares	Mean square	Sum of squares	Mean square			0.05	0.01
Total	107	29078.4	271.7	8552.2	79.9	2757.4	0.174	0.191	—
Between periods	11	23100.0	2100.0	1093.6	89.4	2356.7	0.468	0.576	—
Remainder	96	5978.4	60.3	7458.6	77.7	400.7	0.060	0.200	—

The original data from which Table VII is derived are the production and mortality records of the same pens and contests from which Tables III and IV are derived. While none of the correlations in Table VII are significant, the tendency for production and mortality to move in the

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same direction in the different periods of the year as the total correlation of 0.174 indicates, is not due to any causal association between the two, nor is it even controlled by the same factors as shown by the absence of correlation ( $r=0.060$ ) between the residual variations in production and mortality.

### DISCUSSION

No evidence has been secured in this study which indicates that the "strain of heavy production" shortens the life of the pullet. On the contrary, it has been found that the lighter laying bird is more likely to die than her heavier laying pen mate.

While this study has been concerned only with measuring the relationship between production and mortality and no attempt has been made to establish one as the cause or effect of the other, it seems justified, in view of the figures presented, to conclude that those elements in our modern husbandry practices responsible for most of the heavy egg production which characterises present-day flocks are not responsible for the heavy death-rate. Individual practices which have contributed towards increased production may have unfavourably influenced the death-rate, while individual diseases or causes of death may occur equally in good and poor layers or may even be more prevalent in the better layers. The conclusions presented in this paper apply only to the relationship, in the aggregate, between production and mortality.

### SUMMARY

1. In both Barred Plymouth Rocks and White Leghorns, the birds which died in Canadian Egg-Laying Contests during the year 1929-30 were, on the average, poorer layers than pen mates which survived.

2. The majority of deaths occurred in the lower producing pens.

3. The rise which has occurred in the death-rate since the inception of egg-laying contests in Canada is not connected with the rise in production which has occurred during the same period.

4. During the year 1929-30, although there is a tendency for the rate of production to parallel the rate of mortality throughout the year, a critical analysis of the data shows that the two rates are, in reality, independent.

5. It would appear that practices which contribute towards increased production, in the aggregate at least, contribute also toward health and lowered mortality.

6. While no definite conclusions can be drawn concerning the general

applicability of these findings, there is no reason to suspect that they do not apply to flocks in general.

#### ACKNOWLEDGMENTS

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# THE TRANSPORT OF WATER THROUGH HEAVY CLAY SOILS. I

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(With Three Text-figures)

## INTRODUCTION

SEVERAL attempts have been made in the past to interpret the movement of soil water in terms of permeability, hydrostatic pressure, and viscosity. Theories developed upon this basis, depending as they do upon Poiseuille's equation of fluid flow through tubes, may legitimately be applied only to cases of steady flow through saturated soils. This, in effect, limits their sphere of usefulness to light soils, and in this field they have met with some success in dealing with such problems as the dependence of water-table depth upon separation and depth of drains, in which problems the conditions have been satisfied. They may not be applied to clay soils for two reasons. Firstly, permeability in such soils is a variable function of moisture content, and the saturated condition is rare in practice; and secondly, moisture moves so slowly that the state of steady flow can hardly be set up. The weather conditions determining the state change before it can be attained. Water-table problems are not important in such soils, since they are not known to occur at depths within which the agriculturalist has to work.

In order to deal with clay soils, therefore, it was decided to try some other hypothesis. Since the pressure conditions determining the water flow in such soils are mainly due to surface tension, and not to gravity, they are not easy to deal with theoretically, and consequently the assumption chosen was that the water movement is decided by the moisture concentration gradient, a much more amenable quantity. This means that the water moves according to diffusion equations, *i.e.* the mass of water passing unit cross-section in the soil in unit time is directly proportional to the concentration gradient perpendicular to the section, or, in other words, to the slope of the moisture profile.

This is expressed by the equation

$$\frac{\partial Q}{\partial t} = -k \frac{\partial c}{\partial x}, \quad \dots\dots(1)$$

where  $\partial Q/\partial t$  is the rate of flow in the direction  $x$ ,  $c$  is the moisture concentration expressed as a fraction of the dry matter,  $k$  is the diffusion coefficient, and the negative sign accounts for the fact that the flow is positive when the gradient is negative, so that  $k$  is conventionally positive.

This equation implies that moisture can diffuse upwards as readily as it can downwards, given an equally favourable gradient. This limits its application to heavy clay soils, in which surface tension forces predominate, and in which the equal freedom of upward and downward movement is known to occur(1).

The purpose of this paper is to deduce some of the consequences of the above hypothesis. It will be left to a second paper, dealing with applications to practical cases in the field, to discuss the experimental evidence in support, but it may be remarked here that, upon theoretical grounds, the hypothesis may not be dismissed as improbable. Although the pressure gradient due to surface tension, for a given moisture concentration gradient, is less in a wet soil than in a dry one, yet, as Buckingham pointed out(2), the pores contain more water in the former case and thus provide a path of lower resistance to water flow.

#### THE EQUATION OF CONTINUITY

We obtain the appropriate equation of continuity by considering a semi-infinite medium in which, at any one instant, the moisture content  $c$  is a function of depth,  $x$ , only,  $c$  being expressed as the ratio of mass of water to mass of dry matter in a given volume. Then at time  $t$ ,  $c$  is a function of  $x$  and  $t$

$$c = f(x, t). \quad \text{.....(2)}$$

Now consider a vertical column of unit cross-section, and take an element of length  $dx$  at depth  $x$ . From equation (1), the rate of increase of water,  $\partial q/\partial t$ , in this element of volume is

$$\frac{\partial q}{\partial t} = k \frac{\partial^2 c}{\partial x^2} dx. \quad \text{.....(3)}$$

This increase may also be expressed in terms of the rate of increase of concentration in the elementary volume. Let the weight of dry matter per unit volume of soil be  $\sigma$ , then

$$\frac{\partial q}{\partial t} = \sigma \frac{\partial c}{\partial t} dx. \quad \text{.....(4)}$$

Hence, combining (3) and (4), the appropriate equation of continuity is

$$\frac{\partial c}{\partial t} = \frac{k}{\sigma} \frac{\partial^2 c}{\partial x^2}, \quad \text{.....(5)}$$

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and we assume further, in the following treatment, that  $k/\sigma$  is independent both of depth and time.

### THE SOLUTION OF THE EQUATION

The equation (5) has been very thoroughly investigated in its application to heat problems (3), and the solution is of the form

$$c = P + \sum_n Q_n e^{-\frac{k}{\sigma} m_n^2 t} \cos m_n x + \sum_n R_n e^{-\frac{k}{\sigma} m_n^2 t} \sin m_n x, \quad \dots\dots(6)$$

where  $P$ ,  $Q_n$  and  $R_n$  are arbitrary constants determined by the boundary conditions of special problems.

We may take as one of our boundary conditions that the soil-moisture content is known as a function of  $x$  at some one instant which we may take for convenience as our time zero. Then

$$c(x, 0) = f(x), \quad \dots\dots(7)$$

and will be denoted by  $c_0$ . It may be expanded as a Fourier's series in the well-known form

$$c_0 = A_0 + \sum_n A_n \cos \frac{2\pi n x}{\lambda} + \sum_n B_n \sin \frac{2\pi n x}{\lambda}, \quad \dots\dots(8)$$

where  $\lambda$  is the "wave-length" or space periodicity of the infinitely extending function of which the moisture profile constitutes one, or a half, or a quarter, etc., wave-length, according to the nature of the problem. The coefficients are

$$\begin{aligned} A_0 &= \frac{1}{\lambda} \int_0^\lambda c_0 dx, \\ A_n &= \frac{2}{\lambda} \int_0^\lambda c_0 \cos \frac{2\pi n x}{\lambda} dx, \\ B_n &= \frac{2}{\lambda} \int_0^\lambda c_0 \sin \frac{2\pi n x}{\lambda} dx. \end{aligned} \quad \dots\dots(9)$$

All these coefficients are calculable since  $c_0$  is known. The expansion (8) is clearly a solution of (5) at  $t=0$ , hence we may identify corresponding terms of (6) and (8) and arrive at the solution, for any subsequent time  $t$ ,

$$c_t = A_0 + \sum_n A_n e^{-\frac{k}{\sigma} \left(\frac{2\pi n}{\lambda}\right)^2 t} \cos \frac{2\pi n x}{\lambda} + \sum_n B_n e^{-\frac{k}{\sigma} \left(\frac{2\pi n}{\lambda}\right)^2 t} \sin \frac{2\pi n x}{\lambda}. \quad \dots\dots(10)$$

We must now choose a particular problem to decide the relation between  $\lambda$  and  $l$ , the range of the moisture profile, which should be, rigidly, the whole depth of the clay deposit.

## CASE 1. NO RAINFALL, NO EVAPORATION

The conditions set out above imply that there is a constant quantity of moisture present in the soil, determined by the moisture profile, and we shall investigate the redistribution due to the gradients. The boundary conditions introduced are that there shall be no flow across the two boundaries of the range, *i.e.* flow is zero at  $x=0$  and at  $x=l$ .

The infinite periodic function shown in Fig. 1 satisfies these conditions. The moisture profile is shown as the heavy line between  $x=0$  and

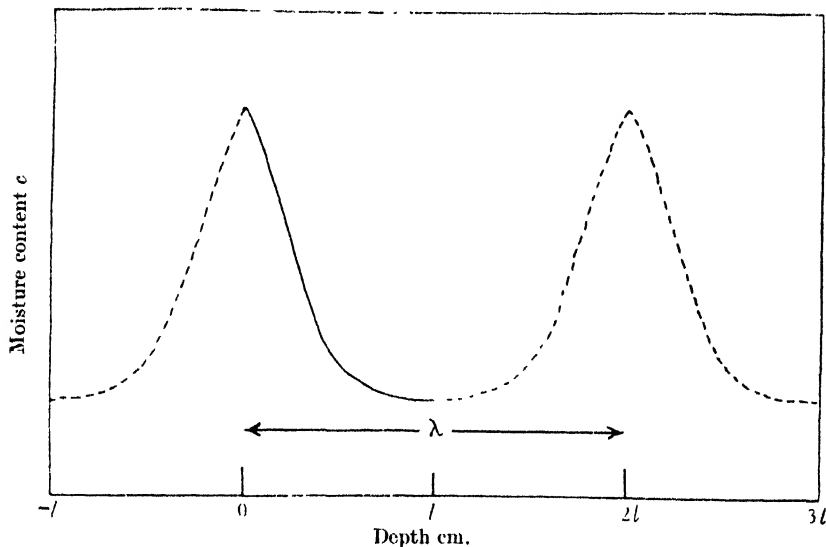


Fig. 1. Moisture profile as part of infinite function, Case 1.

$x=l$ . The fact that the moisture profile outside this range is not as shown by the dotted line is of no consequence. It is merely a device for obtaining the coefficients  $A_0$ ,  $A_n$  and  $B_n$  within the range 0 to  $l$ ; outside this range we are not interested. Physically, however, the redistribution of moisture in our isolated column is exactly the same as if it were part of an infinite column with the periodic moisture profile shown. At infinite time the moisture content would be constant, independent of depth.

We see, then, that  $l$  is half the wave-length  $\lambda$ , and that owing to the symmetry about the ordinate  $x=l$ , the coefficients  $B_n$  are equal to zero, as may be shown by carrying out the appropriate integration in equations (9).

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We have also

$$\begin{aligned} A_0 &= \frac{1}{2l} \int_0^{2l} c_0 dx \\ &= \frac{1}{l} \int_0^l c_0 dx, \end{aligned}$$

since the integration between the limits 0 and  $l$  is equal to that between  $l$  and  $2l$ . Also

$$\begin{aligned} A_n &= \frac{1}{l} \int_0^{2l} c_0 \cos \frac{\pi nx}{l} dx \\ &= \frac{2}{l} \int_0^l c_0 \cos \frac{\pi nx}{l} dx, \end{aligned}$$

since both  $\cos \frac{\pi nx}{l}$  and  $c_0$ , and therefore their product, are symmetrical about the ordinate  $x=l$ .

$$\begin{aligned} B_n &= 0, \\ \text{since } \int_0^l c_0 \sin \frac{\pi nx}{l} dx &= - \int_l^{2l} c_0 \sin \frac{\pi nx}{l} dx. \\ \left. \begin{aligned} A_0 &= \frac{1}{l} \int_0^l c_0 dx, \\ A_n &= \frac{2}{l} \int_0^l c_0 \cos \frac{\pi nx}{l} dx, \\ B_n &= 0, \end{aligned} \right\} \dots\dots(11) \end{aligned}$$

and the correct expansion to use is the half-range cosine series. Physically it is easy to see that, since the flow is zero at  $x=0$  and  $x=l$ , at every instant, the slope of the profile must always be zero at these points. Consequently there can be no sine terms, which provide the slope here. Hence the solution appropriate to the problem is

$$\begin{aligned} c_t &= \frac{1}{l} \int_0^l c_0 dx + \sum_n \left( \frac{2}{l} e^{-\frac{k}{\sigma} \left( \frac{\pi n}{l} \right)^2 t} \int_0^l c_0 \cos \frac{\pi nx}{l} dx \right) \cos \frac{\pi nx}{l} \\ &\dots\dots(12) \\ (n &= 1, 2, 3, 4, \dots). \end{aligned}$$

In practice the integrations have to be carried out laboriously by numerical or graphical methods, since in general  $c_0$  is not an analytical function of depth. A sufficient number of terms must be calculated until  $A_n$  becomes negligible. This may be a large number if the terms converge slowly, but fortunately such convergence is helped, at time  $t > 0$ , by the exponential factors. The range  $l$  should strictly be the whole depth of the clay deposit, but in practice the moisture redistribution is

negligible, within a reasonable time, below a finite depth which is generally about 40 cm., and this depth may be taken, without serious error, as the length  $l$ .

In order to demonstrate the solution the idealised profile of Fig. 2 was taken:

$$c_0 = \left(0.8 - \frac{0.5}{6}x\right) \text{ from } x=0 \text{ to } x=6,$$

$$c_0 = 0.3 \text{ from } x=6 \text{ to } x=40.$$

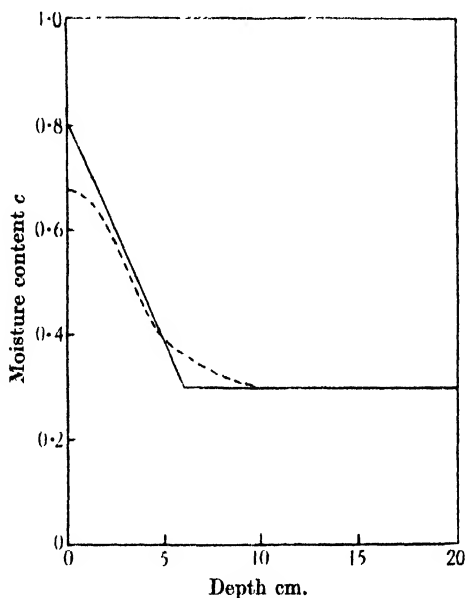


Fig. 2. Redistribution of moisture in the profile.

————— Initial profile      ..... Profile after 1 day

The integrations may then be carried out analytically. To anticipate one of the results of paper II the value of  $k/\sigma$  for the gault clay at Cambridge University Farm is about 2.0 gm. per unit gradient per day.

With these values, Table I, showing  $A_n$  and  $e^{-\frac{k}{\sigma} \left(\frac{\pi n}{l}\right)^2 t}$ , was drawn up for an interval of 1 day. The profile deduced for the end of this period is shown by the dotted line of Fig. 2, and it will be seen that, with the exception of the ends of the sloping part of the profile, redistribution is very slow.

Table I.  $A_0=0.3375$ 

$n$	$A_n$	$e^{-\frac{k}{\sigma} \left(\frac{\pi n}{l}\right)^2}$	$A_n e^{-\frac{k}{\sigma} \left(\frac{\pi n}{l}\right)^2}$
1	0.07360	0.9881	0.07270
2	0.06970	0.9531	0.06640
3	0.06330	0.8976	0.05680
4	0.05530	0.8252	0.04570
5	0.04620	0.7408	0.03420
6	0.03660	0.6492	0.02380
7	0.02740	0.5554	0.01520
8	0.01910	0.4638	0.00887
9	0.01210	0.3783	0.00458
10	0.00676	0.3011	0.00203
11	0.00304	0.2341	0.00071
12	0.00090	0.1776	0.00016
13	0.00005	0.1316	0.00001
14	0.00017	0.0951	0.00002
15	0.00088	0.0671	0.00006
16	0.00182	0.0462	0.00008
17	0.00270	0.0312	0.00008
18	0.00331	0.0205	0.00007
19	0.00354	0.0132	0.00005
20	0.00338	0.0082	0.00003
21	0.00290	0.0050	0.00001
22	0.00222	0.0030	0.00001
23	0.00148	0.0018	0.00000
24	0.00081	0.0010	0.00000

## CASE 2. CONSTANT SURFACE MOISTURE CONTENT

In case 1 we saw that redistribution involved loss of moisture from the upper (wetter) region. Hence maintenance of moisture content at the surface can only be effected by making good the loss by means of, for example, rainfall, and we can calculate the necessary rate of rainfall. This is of interest in connection with the performance of drains because there is good evidence, to be discussed further in paper II, that heavy-land drainage is of the nature of surface run-off of the excess water deposit which the soil cannot accommodate. Thus by considering the maintenance of the surface moisture content at the maximum possible value, the maximum acceptable rainfall can be calculated, and hence the rainfall for which the drains should just run. If the soil is undrained, the excess rain will remain as standing water; the hydrostatic pressure due to this head of water will not increase the rate at which the soil can accept moisture, since the gravitational pressure of even a few inches of water is smaller than the pressures due to surface tension.

In this case we still have the boundary condition of no flow across the lower end of the soil column ( $flow=0$  when  $x=l$ ), and therefore the periodic function will have no slope here and will be symmetrical about the ordinate  $x=l$ . When  $x=0$  the moisture content is constant ( $=m_c$ ) at every instant, and this indicates that the periodic function is sym-

metrical about the *point*  $x=0$ ,  $c=c_m$ . Hence the function is of the type shown in Fig. 3, the periodic distance,  $\lambda$ , being four times the range of the soil profile,  $l$ . It may be observed that the water movement in the actual profile is just that which would occur if the column were part of an infinite column with the periodic moisture profile shown. The rate of flow of water across the boundary  $x=0$ , due to the slope of the profile here, is the quantity we wish to calculate. At zero time, just before rain begins to fall, the moisture content at the surface is naturally less than the maximum possible moisture content  $c_m$ . This means that the periodic function is discontinuous at  $x=0$  and at multiples of  $2l$ , but such a curve is capable of being expressed as a Fourier's series.

If we transfer to the axis  $c=c_m$ , then the curve represents  $(c_0 - c_m)$  as a function of  $x$ , and is symmetrical about the point  $x=2l$ . Hence  $(c_0 - c_m)$  can be expressed as a Fourier's series in which

$$\begin{aligned} A_0 &= \frac{1}{\lambda} \int_0^\lambda (c_0 - c_m) dx \\ &= \frac{1}{\lambda} \left[ \int_0^{1/2\lambda} (c_0 - c_m) dx + \int_{1/2\lambda}^\lambda (c_0 - c_m) dx \right] \\ &= 0, \end{aligned}$$

since the first integral is equal and opposite in sign to the second. Similarly

$$\begin{aligned} A_n &= \frac{2}{\lambda} \left[ \int_0^{1/2\lambda} (c_0 - c_m) \cos \frac{2\pi nx}{\lambda} dx + \int_{1/2\lambda}^\lambda (c_0 - c_m) \cos \frac{2\pi nx}{\lambda} dx \right] \\ &= 0, \\ B_n &= \frac{2}{\lambda} \int_0^\lambda (c_0 - c_m) \sin \frac{2\pi nx}{\lambda} dx. \end{aligned}$$

Since both  $(c_0 - c_m)$  and  $\sin \frac{2\pi nx}{\lambda}$  are symmetrical about the *point*  $x=\frac{\lambda}{2}$ ,  $(c_0 - c_m)=0$ , the integrals over the two halves of the range are of the same sign and augment each other. However, when  $n$  is even, the integrals are separately zero, since in this case  $(c_0 - c_m)$  is symmetrical about the *ordinates*  $x=\frac{\lambda}{4}$ ,  $x=\frac{3\lambda}{4}$ , while the *sines* are symmetrical about the *points*  $x=\frac{\lambda}{4}$ ,  $(c_0 - c_m)=0$  and  $x=\frac{3\lambda}{4}$ ,  $(c_0 - c_m)=0$ . Hence

$$\left. \begin{aligned} B_n &= \frac{2}{4l} \cdot 2 \int_0^{2l} (c_0 - c_m) \sin \frac{\pi nx}{2l} dx \\ &= \frac{2}{l} \int_0^l (c_0 - c_m) \sin \frac{\pi nx}{2l} dx \text{ (for } n=1, 3, 5, 7, \dots) \\ &= 0 \text{ (for } n=2, 4, 6, 8, \dots). \end{aligned} \right\} \dots\dots(13)$$



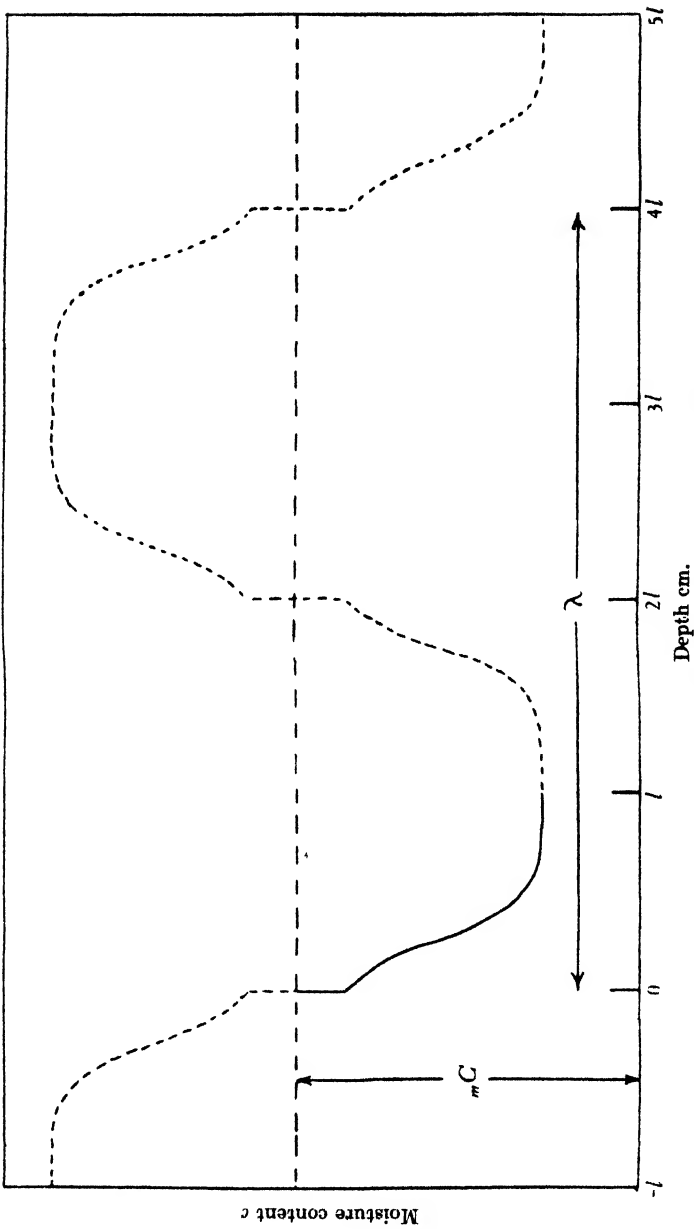


Fig. 3. Moisture profile as part of infinite function, Case 2.

Hence the appropriate expansion is a quarter-range sine series of the form

$$(c_0 - {}_m c) = \sum_n \left( \frac{2}{l} \int_0^l (c_0 - {}_m c) \sin \frac{\pi n x}{2l} dx \right) \sin \frac{\pi n x}{2l}$$

$$(n=1, 3, 5, 7, \dots).$$

The solution of the equation of continuity, satisfying the boundary conditions, is

$$c_t = {}_m c + \sum_n \left( \frac{2}{l} e^{-\frac{k}{\sigma} \left( \frac{n\pi}{2l} \right)^2 t} \int_0^l (c_0 - {}_m c) \sin \frac{\pi n x}{2l} dx \right) \sin \frac{\pi n x}{2l}$$

$$\dots\dots(14)$$

$$(n=1, 3, 5, 7, \dots).$$

This also satisfies, as it should, the boundary conditions  $c_t = {}_m c$  for all values of  $x$  when  $t = \infty$ .

From the expression (14), giving the moisture profile at any time, we can calculate the rate of passage of water across a section of the soil column at any depth  $x$  in either of two ways, both, of course, leading to the same result. We may find the whole mass of water in the column below the section at  $x$  by integrating (14) with respect to  $x$  between the limits  $x$  and  $l$ , and then differentiate the result with respect to time to find the rate at which this increases. The additional water must of necessity have passed the section at  $x$ . Alternatively we may use equation (1), after finding the slope of the profile at  $x$  by differentiating (14) with respect to  $x$ . Adopting the latter procedure, we have, dropping the subscript,  $t$ ,

$$\frac{\partial c}{\partial x} = \sum_n \frac{\pi n}{2l} B_n e^{-\frac{k}{\sigma} \left( \frac{n\pi}{2l} \right)^2 t} \cos \frac{\pi n x}{2l},$$

$$\therefore \frac{\partial q}{\partial t} = -k \sum_n \frac{\pi n}{2l} B_n e^{-\frac{k}{\sigma} \left( \frac{n\pi}{2l} \right)^2 t} \cos \frac{\pi n x}{2l} \dots\dots(15)$$

In practice one measures the total quantity of water,  $q$ , passing in a finite time  $\tau$ , hence by integrating (15) with respect to  $t$  between the limits  $t=0$  and  $t=\tau$  we have

$$q = -\frac{2l\sigma}{\pi} \sum_n \frac{B_n}{n} \left[ 1 - e^{-\frac{k}{\sigma} \left( \frac{n\pi}{2l} \right)^2 \tau} \right] \cos \frac{\pi n x}{2l} \dots\dots(16)$$

$$(n=1, 3, 5, 7, \dots).$$

If we wish to know the maximum quantity of water passing the surface in the first day we put  $x=0$ ,  $\tau=1$  and get the result

$$q_{\max} = -\frac{2l\sigma}{\pi} \sum_n \frac{B_n}{n} \left[ 1 - e^{-\frac{k}{\sigma} \left( \frac{n\pi}{2l} \right)^2} \right] \dots\dots(17)$$

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Any greater quantity of rainfall than this will either run to the drains or remain standing on the surface.

The instantaneous rate of acceptable rainfall is an ever-decreasing amount, being infinitely great during the infinitely short time at the beginning when the slope of the profile is infinitely great. The total acceptable rainfall during the  $r$ th day of the assumed conditions is

$$q_{\max} = -\frac{2l\sigma}{\pi} \sum_n \frac{B_n}{n} \left[ e^{-\frac{k(r-1)}{\sigma} \left(\frac{n\pi}{2l}\right)^2} - e^{-\frac{kr}{\sigma} \left(\frac{n\pi}{2l}\right)^2} \right]. \dots\dots(18)$$

This is less than that of equation (17), since the exponential factor in the latter approaches unity asymptotically as  $n$  increases, and consequently the series converges only as fast as  $B_n/n$  itself. In (18) the exponential factor rises to a maximum and then approaches zero asymptotically; hence the series converges relatively quickly and fewer terms need be taken into account. Thus the theory accounts for the fact that after rainfall sufficiently heavy to flush the drains, a much lighter fall suffices to keep them running.

Physically interpreted, on the beginning of the first day there is a "step" in the moisture profile at the surface, the steep slope of which confers the power of transmitting large quantities of water. The contribution to  $B_n$  for large  $n$  is mainly from the profile near the surface. At a later time, the jump or step fills up, leaving a finite slope. The terms of large  $n$  are reduced by the exponential factor, and the acceptable rainfall is determined mainly by the moisture content at greater depths, being smaller but not so variable with time. During summer, when the surface soil is very dry, it is to be expected that heavy rainfall would be necessary to cause the drains to flush, because of the very high and steep step in the profile near the surface, which would persist for a long time.

### CASE 3. RECOVERY AFTER SURFACE SATURATION

We have seen how the acceptable rainfall during the second day of a drainage period (obtained by putting  $r=2$  in (18)) compares with that during the first. It is of interest to calculate the effect of giving the soil water a period of redistribution without rainfall. Fig. 2 shows that the surface "step" quickly reasserts itself, although the redistribution at greater depths is slow. Hence it might be expected that a short rest period might enable the soil to regain, to a considerable degree, its power of accepting rainfall.

We begin with the profile before rainfall:

$$c_0 = f(x).$$

After  $t$  days of a drainage period the profile is

$$c = {}_m c + \sum_n B_n e^{-\frac{k}{\sigma} \left(\frac{n\pi}{2l}\right)^2 t} \sin \frac{\pi n x}{2l} \quad \text{.....(19)}$$

$$(n=1, 3, 5, 7, \dots).$$

The profile is now allowed a further period to redistribute itself, and consequently we must transform it into a half-range cosine series, appropriate to the solution of case 1. We have

$$c = A_0 + \sum_{\mu} A_{\mu} \cos \frac{\pi \mu x}{l} \quad (\text{for } \mu=1, 2, 3, 4, \dots), \quad \text{.....(20)}$$

where  $A_0 = \frac{1}{l} \int_0^l c dx,$

$$A_{\mu} = \frac{2}{l} \int_0^l c \cos \frac{\pi \mu x}{l} dx.$$

Put  $P_n = B_n e^{-\frac{k}{\sigma} \left(\frac{n\pi}{2l}\right)^2 t},$

and we have  $A_0 = \frac{1}{l} \int_0^l \left( {}_m c + \sum_n P_n \sin \frac{\pi n x}{2l} \right) dx$

$$= {}_m c + \sum_n \frac{2P_n}{\pi n} \quad (\text{for } n=1, 3, 5, 7, \dots),$$

$$A_{\mu} = \frac{2}{l} \int_0^l \left( {}_m c + \sum_n P_n \sin \frac{\pi n x}{2l} \right) \cos \frac{\pi \mu x}{l} dx$$

$$= -\frac{4}{\pi} \sum_n \frac{n P_n}{(2\mu + n)(2\mu - n)}.$$

Hence, after a further period  $t'$ , the profile is

$$c = {}_m c + \sum_n \frac{2P_n}{\pi n} + \sum_{\mu} \left( -\frac{4}{\pi} \sum_n \frac{n P_n}{(2\mu + n)(2\mu - n)} \right) e^{-\frac{k}{\sigma} \left(\frac{\pi \mu}{l}\right)^2 t'} \cos \frac{\pi \mu x}{l}$$

$$(\mu=1, 2, 3, 4, \dots; n=1, 3, 5, 7, \dots), \quad \text{.....(21)}$$

or  $c = Q + \sum_{\mu} R_{\mu} \cos \frac{\pi \mu x}{l} \quad \text{.....(22)}$

This in turn is the profile at the beginning of the second period  $t''$

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of drain flushing, and must be transformed back into the quarter-range sine series appropriate to case 2.

$$c = {}_m c + \sum_{\nu} S_{\nu} e^{-\frac{k}{\sigma} \left( \frac{\nu \pi}{2l} \right)^2 t''} \sin \frac{\pi \nu x}{2l} \quad \dots\dots(23)$$

( $\nu = 1, 3, 5, 7, \dots$ ).

The acceptable rainfall may be calculated from this as in case 2. It may be shown by a process similar to that used for deducing  $A_{\mu}$ , that

$$S_{\nu} = \frac{4}{\nu \pi} (Q - {}_m c) - \sum_{\mu} \frac{4R_{\mu}}{\pi} \frac{\nu}{(2\mu + \nu)(2\mu - \nu)} \quad \dots\dots(24)$$

( $\mu = 1, 2, 3, 4, \dots$ ;  $\nu = 1, 3, 5, 7, \dots$ ).

Application of this result to a practical case is extremely laborious, and as an example will be dealt with in the following paper, it is not proposed to treat an idealised profile here.

### OTHER CASES

As an example of a slight difference in the boundary conditions which renders the problem extremely difficult, we may take the case of constant rate of flow across the surface, *i.e.* constant rainfall or constant evaporation. The exact solution is not obtainable, since one cannot choose a periodic function, satisfying the differential equation, such that the slope at one fixed point is constant, unless the constant is zero. This reduces it to the case of pure redistribution dealt with as case 1. Approximations thus have to be introduced. This difficulty is not serious, since very steady conditions of rainfall are not common in our climate.

### SUMMARY

Assuming a uniform heavy soil, without tilth, the water movement in which obeys diffusion laws, expressions have been deduced for four quantities, starting with a known moisture profile.

(a) The moisture profile at any subsequent time in the absence of rainfall or irrigation (neglecting evaporation, transpiration, and any other source of loss of moisture).

(b) The moisture profile at any subsequent time during such rainfall as causes flushing of the drains.

(c) The minimum rainfall necessary to cause flushing of the drains (maximum acceptable rainfall).

(d) The acceptable rainfall after a period of recovery following the previous drainage period.

The author wishes gratefully to acknowledge the benefits of discussion with Dr J. Wishart and Dr H. M. Taylor, and to express his appreciation of the sustained interest which Prof. F. L. Engledow has taken in the problem.

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# THE TRANSPORT OF WATER THROUGH HEAVY CLAY SOILS. II

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(With Four Text-figures)

## INTRODUCTION

IN a preceding paper<sup>(1)</sup> were deduced some of the consequences of assuming that water movement in a heavy clay soil is a process akin to diffusion. The only idealisation of the medium was the assumption that the ratio of  $k$ , the diffusion coefficient, to  $\sigma$ , the apparent specific gravity of the dry matter in the soil, is constant, independent of depth, and we shall see that this is justified by the experimental evidence. As long ago as 1907, Buckingham<sup>(2)</sup> showed that the quantity of moisture moving in a given time from a block of wet soil to a drier block in contact with it was dependent mainly on the difference between the respective water contents, and that there was no definite dependence on the actual water contents themselves. Veihmeyer<sup>(3)</sup>, in 1927, placed blocks of wet soil between blocks of drier soil, arranged in a vertical column. Subsequent moisture profiles were typical of a redistribution obeying diffusion laws, and were nearly the same both for upward and downward movement. He demonstrated furthermore that the movement of the water in these conditions was extremely slow, and that the rate of movement was related to the difference in moisture content between the dry and wet soil. Unfortunately insufficient numerical data were given to apply diffusion theory to Veihmeyer's experimental results.

However slow the movement of water through heavy soils may be, as compared with light soils, it is apparent from continuous observations of incidence of rain, changes in moisture profile, and details of outfall performances, that over a considerable range of conditions it is sufficient to prevent drainage from taking place even after markedly heavy individual falls of rain. During the average winter of this neighbourhood, however, conditions frequently obtain which are favourable to drain flushing. Some of these conditions are critically examined in this paper. The constitution of the soil profile on which these studies are based and

the salient features of the outfall records have already been described elsewhere (4).

When we attempt to apply our theory to field soils we are forced to the conclusion that the calculated rate of moisture redistribution, acceptable rainfall, etc., are really lower limits. For example, in calculating the acceptable rainfall we assumed that there is a maximum possible water content which the rainfall just maintains at the surface. In

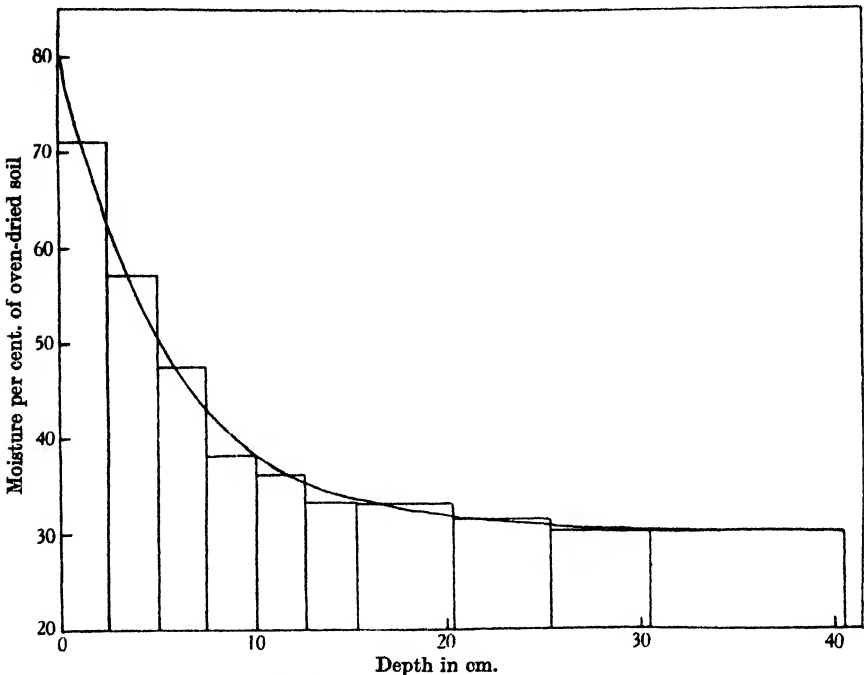


Fig. 1. Typical moisture profile in gault clay (grassland).

practice, with a finite depth of tilth in which water may move fairly freely, we have a layer overlying the uniform medium, and in which this maximum moisture content can be maintained. The water necessary to raise this layer to the required moisture content should be added to the calculated acceptable rainfall for the first day. Once the moisture content is attained, the practical and theoretical conditions should more nearly coincide, so that the calculated acceptable rainfall for the second day of a drainage period should agree more closely with observation than that for the first day. Then, when we deal with redistribution, we have to neglect evaporation and transpiration, which probably remove water



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from the root range at a rate comparable with the rate of downward diffusion. For these reasons our experiments are confined mainly to old grassland in winter, the disturbing factors thus being reduced to a minimum. All the field moisture profiles and outfall records used in this paper were obtained from Youngman's Pasture on the Cambridge University Farm. This field is old grassland laid up in high-backed lands, approximately 30 ft. wide, with mole drains drawn along the bottoms of the intermediate furrows.

The moisture profiles were obtained from cores taken by means of augers, the first 12 in. in 1-in. sections, from 12 to 28 in. in 2-in. sections, and at greater depths in 6-in. sections. The samples were dried to constant weight in a steam oven. A typical example is given in Table I and Fig. 1. This is given because, although it was taken early, before the above sectioning was decided upon, it is one of the most reliable, being taken during good conditions for calculating  $k/\sigma$  and on a known site (a furrow). The profile used was the smoothest curve that could be drawn through the experimentally obtained "stepped" figure.

Table I. *Moisture profile, furrow, November 6, 1931*

Depth in.	Moisture content (fraction of dry matter)
0-1	0.71
1-2	0.572
2-3	0.477
3-4	0.381
4-5	0.361
5-6	0.332
6-8	0.331
8-10	0.315
10-12	0.304
12-16	0.302
16-20	0.299
20-24	0.316
24-28	0.310

### THE COEFFICIENT OF DIFFUSION

The first quantity to be determined is the ratio  $k/\sigma$ , and the method used was based as closely as possible on that used for measuring the diffusion of a salt through its solvent, *i.e.* by observation of the concentration in a column at various heights and times. There is at present no satisfactory method of determining the moisture profile in a clay soil without spoiling the site for subsequent measurements, and consequently it is not possible to keep a single column under observation. Accordingly the records of the past four years were searched for pairs of profiles taken within a short time of each other, and yet showing considerable

change. These, of course, all occurred in winter conditions, in which rainfall maintained steep gradients giving rise to considerable moisture movement. It is important that the time interval should be short, since we assume steady conditions between the taking of the two profiles, and a considerable movement of moisture is desirable in order to make the experimental error small in comparison. In some cases the sites of the profiles were known and matched (*e.g.* ridge profile compared with

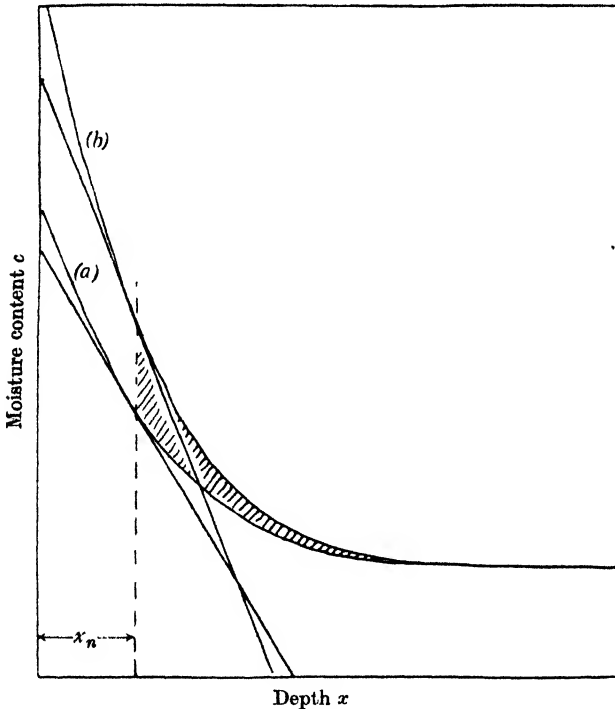


Fig. 2.

ridge, furrow with furrow), giving the most reliable results of all our observations.

The smoothed-out profiles were drawn on a large scale and continued down to the region of no change (to 40 cm.). The slopes at various depths  $x_1$ ,  $x_2$ , etc., were measured, as were the areas contained between the two curves beyond these depths (see Fig. 2). The area beyond  $x_n$ , for example, is proportional to the mass of water passing  $x_n$  in the period between the taking of the two profiles, and this passage of water is a consequence of the slope of the profile at  $x_n$ . The average slope at  $x$  was plotted

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against the area beyond  $x$  for various values of  $x$ , as shown in Fig. 3. If the ratio  $k/\sigma$  is constant, the result should be a straight line passing through the origin, of slope proportional to  $k/\sigma$ . Mass of water,  $Q$ , passing  $x$  is

$$\sigma \int_x^{\infty} (c_2 - c_1) dx,$$

where  $c_2$  refers to the later profile and  $c_1$  to the earlier. Therefore  $Q = \sigma A$ , where  $A$  is the area between the curves beyond  $x$ . If  $t$  is the interval of time between the two profiles, and we assume constant rate of flow, then

$$\frac{\partial Q}{\partial t} = \frac{Q}{t} = \frac{\sigma A}{t},$$

$$\therefore \frac{\sigma A}{t} = -k \frac{\partial c}{\partial x}.$$

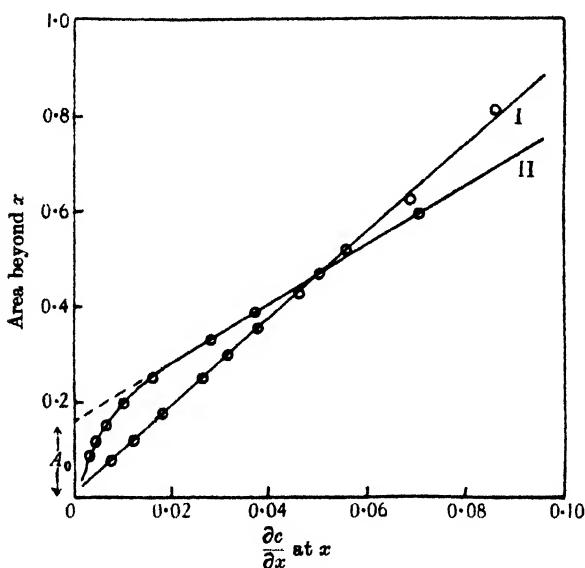


Fig. 3. Relation between rate of passage of water and profile slope causing it.

Hence if  $A$  is plotted as ordinate against  $\partial c/\partial x$  as abscissa, the curve is a straight line of slope  $-kt/\sigma$ , passing through the origin.

In practice good straight lines were obtained over the greater part of the curve, two of which are shown in Fig. 3. Usually, however, they did not pass through the origin. For small profile slopes the curves departed from linearity to pass through the origin. The intercept  $A_0$  of Fig. 3 may represent water moving due to gravity, since it is moving in the absence of a concentration gradient, but it is more probably the

result of the present necessarily crude experimental technique, and the pairing of profiles taken from different sites. The slopes of the curves gave values of  $k/\sigma$  ranging from 1 to 3 c.c. per unit slope per day,  $\sigma$  being expressed as gm. per c.c. Mostly the values, including the most reliable ones, were in the neighbourhood of 2, the mean value, and until the technique has been developed we shall use this value to insert in the diffusion equations.

#### THE MAXIMUM ACCEPTABLE RAINFALL

The ordinary concepts of percolation of surplus moisture through soil, the formation and rise of a water table, and the run-off *via* the drains after the water table reaches their level, break down in the case of drainage of heavy soils. In light soils the examination of outfall records shows them to fit this idea of events, but in the case of heavy soils the run-off has very different characteristics. Flodkvist (5) first advanced the theory that surplus water reached the drains without traversing the body of the subsoil and by a path different from that envisaged by the percolation theory. Outfall records for drainage systems in heavy soils are characterised by very rapid changes from dryness to heavy flow, by the attainment of rates of flow outstandingly greater than those associated with permeable soils, and by subsequent rapid decreases to low rates of flow or even dryness once more. Essentially, in order to explain these facts, Flodkvist suggested that the outfall water is that part of the rainfall in excess of the amount which can percolate downwards through the soil at any moment, and which therefore flows by gravity down the slope of the surface, in the highly permeable tilth layer. A considerable amount of indirect evidence in general support of this explanation has been accumulated in the case of the heavy gault soil of the Cambridge University Farm. An obvious complement to this idea, and one further suggested by the failure of drains to run after heavy individual falls of rain in summer conditions, is that of a "maximum acceptable rainfall" determined by the moisture profile.

If all the moisture profiles obtained in this investigation are examined and grouped as in Fig. 4 according to whether

- (a) the mole channels were definitely in action (marked flow),
- (b) they were just wet (slight efflux),
- or (c) they were dry (no efflux),

it is apparent that the range of moisture content in the profile for condition (a) overlaps slightly that for condition (c), but that the range for condition (b) overlaps both (a) and (c) considerably. It would appear that over a certain range of moisture content, in actual fact the overlap

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of (a) and (b), it is possible for a marked efflux to occur when the profile is in a less moist condition than on another occasion when there is no efflux. For this to be possible suggests that rain may be falling on the surface more rapidly than it can move downwards through the soil profile, and that the excess moves laterally through the more permeable surface soil to the mole slits and channels. With a moister profile the rain may be falling on the surface less rapidly than it can diffuse down-

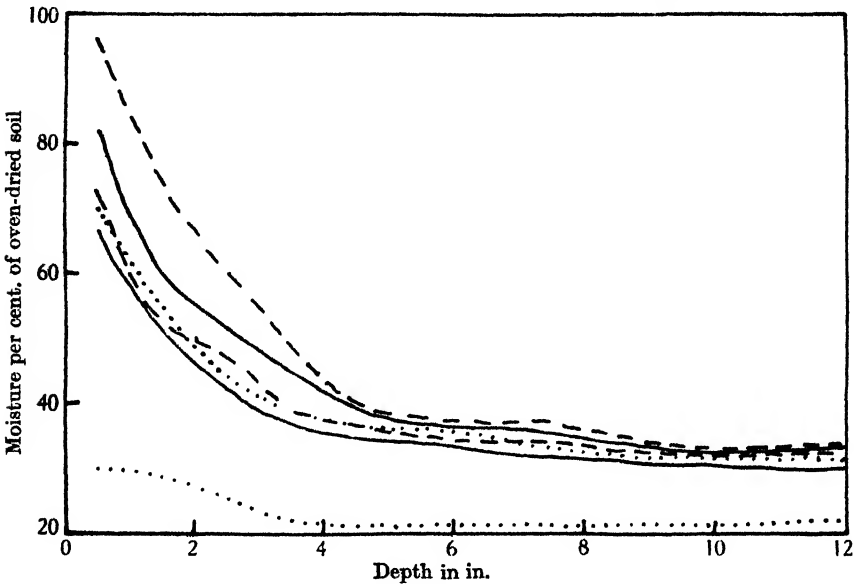


Fig. 4.

Limits of moisture profiles in association with drains running freely	-----
"          "          "          "          just wet	-----
"          "          "          "          dry	.....

wards and no efflux takes place, although initially the profile may be more moist than in the former set of circumstances.

With the above mechanism of drainage in mind, the ideal data for testing the conclusions of case 2 of paper I would be a number of moisture profiles varying very little from one another, obtained upon different occasions when the rainfall differed in intensity over a wide range. The occasions could then be divided into two groups, one in which the rainfall sufficed to make the drains run, and the other in which no efflux was observed. The rainfall at the dividing line should be equal to the calcu-

lated maximum acceptable rainfall for that particular moisture profile. Unfortunately the period for which records were available included the drought years 1933-4, and records of outfalls were not sufficiently extensive for this to be done. Calculations have been made upon two occasions on the basis of three separate moisture profiles. In November of 1931 moisture profiles both on a ridge and in an undrained furrow were available, and were taken just before a fall of 0.4 in. of rain which caused a drain efflux. Profiles were taken again just afterwards, and these were of use in calculating  $k/\sigma$ . This case is interesting in showing that the ridge, being drier, is able to accept a greater rainfall than the furrow. The drains should, however, run at the lower rainfall (for the furrow), since the surface flow of excess moisture is downhill from regions of drier to regions of wetter profile.

On the second occasion, in April 1934, the soil was exceedingly dry, and a heavy fall of 0.45 in. of rain was experienced, followed a week later by a heavier one of 0.6 in. The drains did not, however, run, although they were possibly on the point of doing so since mole drains in neighbouring arable land ran.

In Table II are given the moisture profiles corresponding to the two dates, and in order to calculate the terms  $B_n$  in Table III by the method of case 2, paper I, the maximum surface water content,  $_m c$ , was estimated at 1.2, expressed as a fraction of the dry soil matter. Profiles during rainfall have been known to exceed 1.0 in the top inch, and extrapolate to about the assumed value of 1.2. On the basis of a true specific gravity of 2.7 for the dry matter, this yields a value of 0.67 for  $\sigma$ , the apparent specific gravity. Overestimation of  $_m c$  leads to underestimation of  $\sigma$ , and *vice versa*. Very accurate estimation is not therefore necessary, since overestimation of  $\Sigma B_n$  follows upon overestimation of  $_m c$ , and the product of  $\sigma$  and  $\Sigma B_n$  in equations (17) and (18) of paper I is not therefore very sensitive to errors of  $_m c$ .

It will be seen that even up to the fifty-first term (the even terms being zero) the contributions of  $B_n$  during the first day are not negligible, and are converging but slowly. Fortunately an approximation may be made avoiding the necessity of laboriously calculating a large number of terms beyond the fifty-first.

The function  $(_m c - c_0) \sin \frac{n\pi x}{2l}$ , which has to be integrated to give us  $B_n$ , is an oscillating one, and for large  $n$  the curve  $(_m c - c_0)$  is approximately a straight line over one period of oscillation. Hence the integral is halved in value when  $n$  is doubled, and the term  $B_n/n$  is therefore, at

Table II. *Moisture profiles*

Depth <i>z</i> cm.	Ridge 6. xi. 31 (A)	Undrained furrow 6. xi. 31 (B)	16. iv. 34 (C)
0	0.750	0.810	0.391
1	0.630	0.722	0.391
2	0.550	0.658	0.391
3	0.489	0.604	0.390
4	0.447	0.554	0.388
5	0.418	0.512	0.379
6	0.397	0.476	0.358
7	0.380	0.446	0.339
8	0.366	0.422	0.323
9	0.354	0.401	0.314
10	0.345	0.384	0.308
11	0.338	0.370	0.305
12	0.332	0.358	0.305
13	0.326	0.350	0.305
14	0.322	0.343	0.305
15	0.319	0.338	0.305
16	0.316	0.332	0.304
17	0.314	0.328	0.304
18	0.311	0.325	0.303
19	0.309	0.321	0.303
20	0.308	0.318	0.303
21	0.306	0.316	0.302
22	0.305	0.314	0.302
23	0.304	0.313	0.302
24	0.303	0.311	0.301
25	0.303	0.309	0.301
26	0.302	0.307	0.300
27	0.302	0.306	0.300
28	0.301	0.305	0.300
29	0.301	0.304	"
30	0.300	0.304	"
31	"	0.303	"
32	"	0.302	"
33	"	0.302	"
34	"	0.301	"
35	"	0.300	"
36	"	"	"
37	"	"	"
38	"	"	"
39	"	"	"
40	"	"	"

$n=101$ , only one-quarter of the value at  $n=51$ . Using the average value and multiplying by the number of terms in the group (25, since even terms are missing), we can make an estimation of the contribution of the group. All the terms may in this way be split up into groups, from  $n=51$  to  $n=101$ , from  $n=101$  to  $n=201$ , and so on, and each group makes a contribution equal to half that of the preceding one. Hence we have an infinite geometric series of common ratio 0.5, the sum of which is equal to twice the value of the first term. This sum is the quantity called "contribution 51 -  $\infty$ " in Table III. Column 3 of Table III gives the terms necessary to calculate the acceptable rainfall on the

second day, and it will be seen that the rapid convergence due to the exponential factor renders the foregoing approximation unnecessary.

In a similar way the results for the remaining two profiles were calculated, and the whole collected together in Table IV.

Table III. *Ridge profile (A)*

$n$	$-\frac{B_n}{n} \left( 1 - e^{-\frac{k}{\sigma} \left( \frac{n\pi}{2l} \right)^2} \right)$	$-\frac{B_n}{n} \left( e^{-\frac{k}{\sigma} \left( \frac{n\pi}{2l} \right)^2} - e^{-\frac{2k}{\sigma} \left( \frac{n\pi}{2l} \right)^2} \right)$
1	0.00342	0.00342
3	0.00307	0.00298
5	0.00272	0.00251
7	0.00239	0.00206
9	0.00218	0.00171
11	0.00191	0.00136
13	0.00162	0.00098
15	0.00160	0.00082
17	0.00136	0.00057
19	0.00118	0.00040
21	0.00105	0.00028
23	0.00097	0.00020
25	0.00091	0.00014
27	0.00076	0.00008
29	0.00070	0.00007
31	0.00061	0.00002
33	0.00052	—
35	0.00047	—
37	0.00043	—
39	0.00038	—
41	0.00036	—
43	0.00032	—
45	0.00031	—
47	0.00028	—
49	0.00026	—
51	0.00025	—
$\sum_{n=1}^{\infty}$	0.03003	—
Contribution 51 to $\infty$	0.00800	—
$\sum_{n=1}^{\infty}$	0.03803	0.01760

$$\begin{aligned} \text{Acceptable rainfall: first day} &= -\frac{2l\sigma}{\pi} \sum_{n=1}^{\infty} \frac{B_n}{n} \left( 1 - e^{-\frac{k}{\sigma} \left( \frac{n\pi}{2l} \right)^2} \right) \\ &= 0.65 \text{ cm. or } 0.26 \text{ in.} \\ \text{second day} &= 0.30 \text{ cm. or } 0.12 \text{ in.} \end{aligned}$$

Table IV

Date	Rainfall in.	Acceptable rainfall (calculated) in.	Remarks
6. xi. 31 (ridge)	0.40	0.26 (1st day) 0.12 (2nd day)	Drains ran
6. xi. 31 (furrow)	0.40	0.21 (1st day) 0.10 (2nd day)	Drains ran
16. iv. 34	0.43	0.35 (1st day) 0.14 (2nd day)	Drains did not run



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On November 6, 1931, the drains did not run very strongly, so that the real acceptable rainfall was probably very near 0.40 in., *i.e.* the calculated values, as expected, are too low. On none of these occasions did rain fall during two or more consecutive days, so that the more satisfactory test of the second day's acceptable rainfall could not be directly applied.

In the case of the April profile, therefore, the rate of soil recovery was worked out, as in case 3 of paper I, for recovery periods of 1 day and 5 days. This very laborious calculation was not repeated for the other profiles, but estimates were made as shown in Table V.

Table V. *April 14, 1934*

Recovery period days	Subsequent acceptable rainfall in.	Estimated acceptable rainfall for recovered ridge profile, 6. xi. 31 in.
0 (from Table IV)	0.14	0.12
1	0.19 (approx. $\frac{1}{4}$ recovered)	0.16
5	0.27 (approx. $\frac{3}{4}$ recovered)	0.22
	0.35 when fully recovered	0.26 when fully recovered

In April 1934, following the fall of 0.4 in. rain, a further 5 days elapsed before another fall, of 0.2 in., and 7 days before a heavy fall of 0.63 in. After a further interval of 1 day, another 0.4 in. fell. On none of these occasions were the drains observed to be functioning, although from the behaviour of some mole drains in neighbouring arable land it is probable that they were on the point of flushing. With these long periods elapsing since the profile was taken, the calculated values cannot by anything but rough estimates, but it is clear that they are underestimates.

In November 1931, after an interval of 2 days, a fall of 0.21 in. of rain caused the drains to run fairly strongly again. Records of the outfall rates being available, the excess rainfall could be calculated, and hence the observed acceptable rainfall could be compared with the estimated value in Table V. The outfall rate rose from zero to 100 gal./acre/hour and fell to 22 gal./acre/hour during the day, the approximate total during the day being 1392 gal./acre. This corresponds to a rainfall of 0.06 in., leaving 0.15 in. absorbed by the soil. By interpolation from column 3, Table V, the calculated acceptable rainfall after a recovery period of 2 days is 0.18 in., which is in fair agreement with observation considering

the approximate nature of the present treatment. Subsequent falls of 0.12 and 0.13 in. on consecutive days caused the drains to run moderately, so that agreement with Table IV may be considered fairly satisfactory.

#### DISCUSSION

We have seen that if we base our theory of water movement in clay soils on diffusion equations, we can reach a fairly satisfactory agreement with practical experience under certain conditions, namely that the soil shall be fairly moist (winter conditions) and that the drain efflux shall not be the first of the season. For summer conditions the calculated acceptable rainfall is considerably underestimated (being, say 50 per cent. of the observed) while there is somewhat less but still serious underestimation, in winter conditions, for the first day's acceptable rainfall of a drainage period following a dry period.

The true soil may, to a first approximation, be considered as a uniform medium (subsoil) of the type envisaged in the treatment of paper I, covered with a permeable tilth layer of finite thickness which can take up the maximum water content possible. The whole of this layer must first attain this water content and be maintained at it before drain efflux will begin. From a knowledge, on one occasion, of the unoccupied pore space and the excess of observed over calculated acceptable rainfall, we can calculate, for any other occasion, the thickness of the tilth layer and the correction which must be applied to the calculated acceptable rainfall. Beyond saying that in this way, from calculations based on the 1931 winter conditions, the behaviour in April 1934 can be accounted for, we shall not proceed further, because the most satisfactory solution of the problem is a more detailed investigation of the moisture profile near the surface. It is intended to carry this out next winter.

It is possible that the theory will never be brought satisfactorily into line with summer conditions, because the dryness of the soil produces contraction and therefore fissures which, in effect, increase the surface available for maximum moisture content by a large and incalculable amount. It is also possible that diffusion of water is only important for relatively rapid movement, such as is caused by the steep gradients maintained by heavy rainfall. The slow redistribution during dry weather may be small compared with removal by evaporation and transpiration.

A few of the many points which call for future investigation may be mentioned here. First, it has been tacitly assumed that the normal or equilibrium profile, which the soil moisture would exhibit if given a

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sufficiently long time of undisturbed rainless conditions to reach equilibrium, would indicate constant moisture content irrespective of depth. This may be untrue in nature, since variations, with depth, of organic matter and prevalent mineral particle size would result in moisture content dependent on depth; there is no evidence since the required long period of the correct conditions never occurs. The diffusion law may have to be restated tentatively as follows. The rate of passage of water at any point in the profile is proportional to the difference between the profile slope and the slope of the normal profile at the point. The normal profile can only be found by isolating and hermetically sealing a soil column in the laboratory, care being taken to preserve structure, and allowing the water to reach its equilibrium distribution. It is improbable that, in the calculation of acceptable rainfall, any serious error is incurred by ignoring the possibility of a characteristic normal profile, for the condition of heavy rainfall maintains gradients which must be steep compared with those of the true equilibrium profile.

Methods of taking the moisture profile are being developed which do not involve destruction of the site, so that detailed observation may be made at small time intervals on a single soil column *in situ*. The electrical methods so far described by various authors have been found inadequate, while the porous pot method involves disturbance of the soil during installation of the apparatus, and this is very likely to disturb the moisture profile locally, just where it is being measured.

It is at first sight somewhat surprising that  $k/\sigma$  is so constant down the soil profile. Mechanical analysis, however, shows that the soil profile itself does not change greatly within the range of depths effective in the problems dealt with in these papers(6). Near the surface, where the greatest variation is to be expected, the moisture profiles are not known very accurately. The method of sampling gives the average moisture content in each inch of depth, so that, at the surface where the profile is steepest, it is in the nature of an extrapolation (see Fig. 1). A more detailed investigation of the surface layer during the next drainage season should clear up this point, and it should be possible to say then whether or not the ratio  $k/\sigma$  is characteristic of a given soil, or whether it is rather the ratio of the "diffusing" to "gravitating" water which indicates the heaviness or lightness of a soil.

It is a pleasure to acknowledge the help and co-operation afforded by Prof. F. L. Engledow and Mr W. S. Mansfield throughout the course of the investigations.

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# COMPOSITION, DIGESTIBILITY AND NUTRITIVE VALUE OF SAMPLES OF GRASSLAND PRODUCTS

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(With Three Text-figures)

THE technique adopted in measuring the digestibility and feeding value of foodstuffs for use on the farm has been published in an earlier paper<sup>(1)</sup>.

The tables at the end of this paper give full details of the data obtained on a large number of samples of agricultural produce examined at this station. For convenience, they have been classified under five headings which are further subdivided.

- (1) Fresh grass: (a) Cut for conservation at different stages of growth.  
(b) Pasture grass under grazing conditions.
- (2) Hay: (a) Poor.  
(b) Medium.  
(c) Good.
- (3) Silage: (a) From pits. Made from grassland herbage.  
(b) From towers. Made from grassland herbage.  
(c) From towers. Made from silage crops.  
(d) From stacks. Made from grassland herbage.  
(e) From stacks. Made from forage crops.  
(f) Potato silage.
- (4) Artificially dried fodder: (a) Grass.  
(b) Clover and lucerne.
- (5) Miscellaneous: (a) Marrow-stem kale.  
(b) Sugar-beet tops and leaves.  
(c) Pea, bean and lentil shells.  
(d) Wheat and barley.  
(e) Mangolds.  
(f) Dreg meal.  
(g) Potatoes.

## FRESH GRASS

Most of the samples examined have been in connection with grass conservation experiments. Under these conditions the growth is relatively even over the whole field, and there is a more or less regular increase in starch equivalent and protein equivalent content with increasing crude protein.

The curve in Fig. 1 is based on the data accumulated, and will serve as a guide to the nutritive value of a crop of grassland herbage containing a fair proportion of clover. It is not applicable to "seeds" mixtures. An estimation of the crude protein content of the crop will give average values for the starch and protein equivalents by interpolation in the curves for these two constituents.

*Grass grazed at an advanced stage of maturity.* The second set of figures is included more as a matter of interest than practical import. They were obtained from a pasture grazed at an advanced stage of maturity by cows. The samples obtained by scythe for the metabolism trials include all herbage offering, and the result is seen in the high number of samples of low protein content. In point of fact, at such times the cows were grazing selectively, and a sample specially picked from leafy material showed a protein content of 25 per cent. in the dry matter. These results are indicative of the difficulty which obtains when it is desired to take samples which will be comparable with the herbage actually grazed by the stock at any particular time. It is only when closely grazed pasture is sampled (Sample No. 11) that a high protein content is obtained, and that it can be assumed that the material is representative of the herbage eaten by the stock when first turned in. The figures for the herbage at a stage of advanced maturity are actually an average of the high-quality material selected by the first line of grazing stock—dairy cows in this case—and the poor-quality stemmy material which is eaten by the less-productive stock put in to clean up the pasture. On pasture grazed extensively and where the more stemmy material remains ungrazed, it is well-nigh impossible to obtain any direct measure of the nutritive value of the herbage as actually eaten.

## HAY

The most striking feature of the hay figures is the low crude protein content in the dry matter. The "poor" hays are thus classified by virtue of their crude protein content. Actually they would have been described by the farmer as average samples of good meadow hay, whilst those with

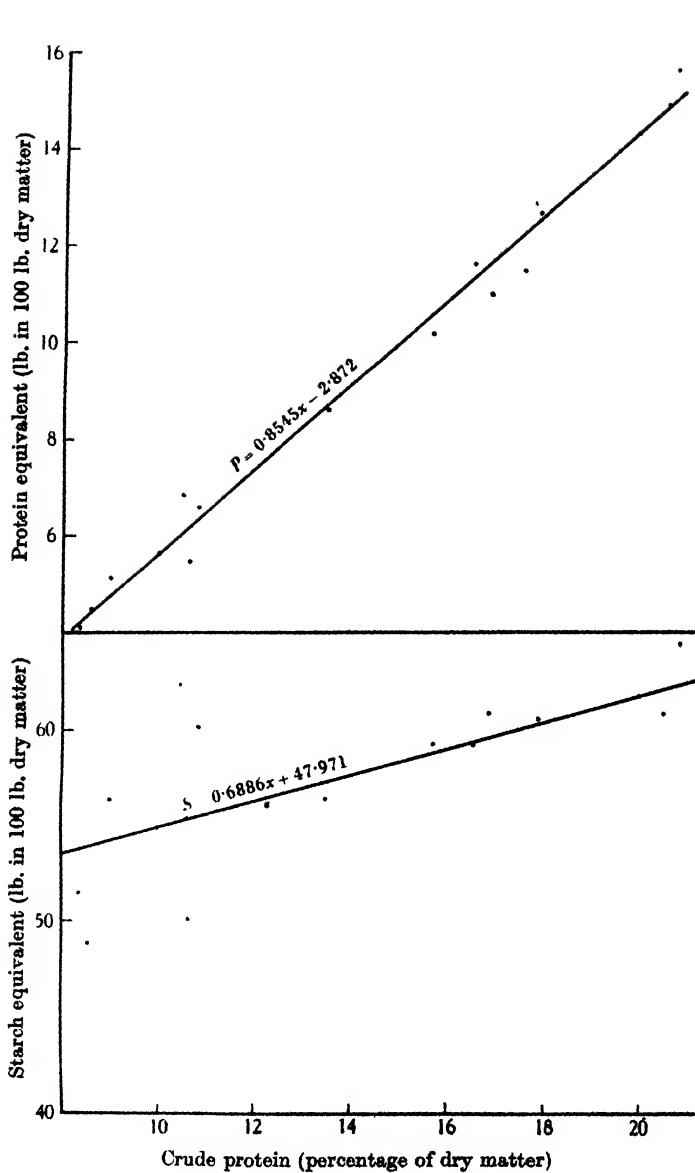


Fig. 1. Fresh grass.

over 8 per cent. of crude protein (medium hay) would have been classed as above average. The good hays were all cut some 3-4 weeks before normal hay-making time—towards the end of May—and show a marked improvement in composition over the ordinary hay, though the yield of dry matter would be lighter. They point clearly to the desirability of early cutting of hay. The average value of all the samples of ordinary hay is contrasted with those of the early hay in Table I.

Table I. *Starch equivalent and protein equivalent content of ordinary hay (ten samples) and early cut hay (five samples). (Stated as percentages of the dry matter.)*

	Crude protein %	Starch equivalent lb. per 100 lb.	Protein equivalent %
Ordinary hay	7.26	38.3	3.03
Early cut hay	12.77	47.9	6.78

The figures show that the ordinary hay samples had a starch equivalent similar to the value usually ascribed to average meadow hay, but that the protein equivalent value is lower than is usually assumed.

The most noticeable feature of the hay figures is the high digestibility of the fibre as compared with figures in the literature. Kellner<sup>(2)</sup> gives the following average values (Table II) for meadow hay.

Table II. *Digestibility coefficients of hay quoted by Kellner<sup>(2)</sup> and average values of figures obtained at Jealott's Hill*

	Organic matter		Crude protein		Ether extract	
	Kellner	These exps.	Kellner	These exps.	Kellner	These exps.
Hay: rich in protein	67	71	65	58	57	43
medium in protein	61	64	57	51	51	35
poor in protein	56	62	50	45	49	32

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	N-free extractives		Crude fibre	
	Kellner	These exps.	Kellner	These exps.
Hay: rich in protein	68	71	63	78
medium in protein	64	66	59	67
poor in protein	59	64	55	66

It is difficult to account for this difference otherwise than by the fact that the fields cut for hay in the authors' determinations had all been treated with nitrogen, and the type of growth was more leafy than is normal. It is proposed to carry out a series of metabolism trials during



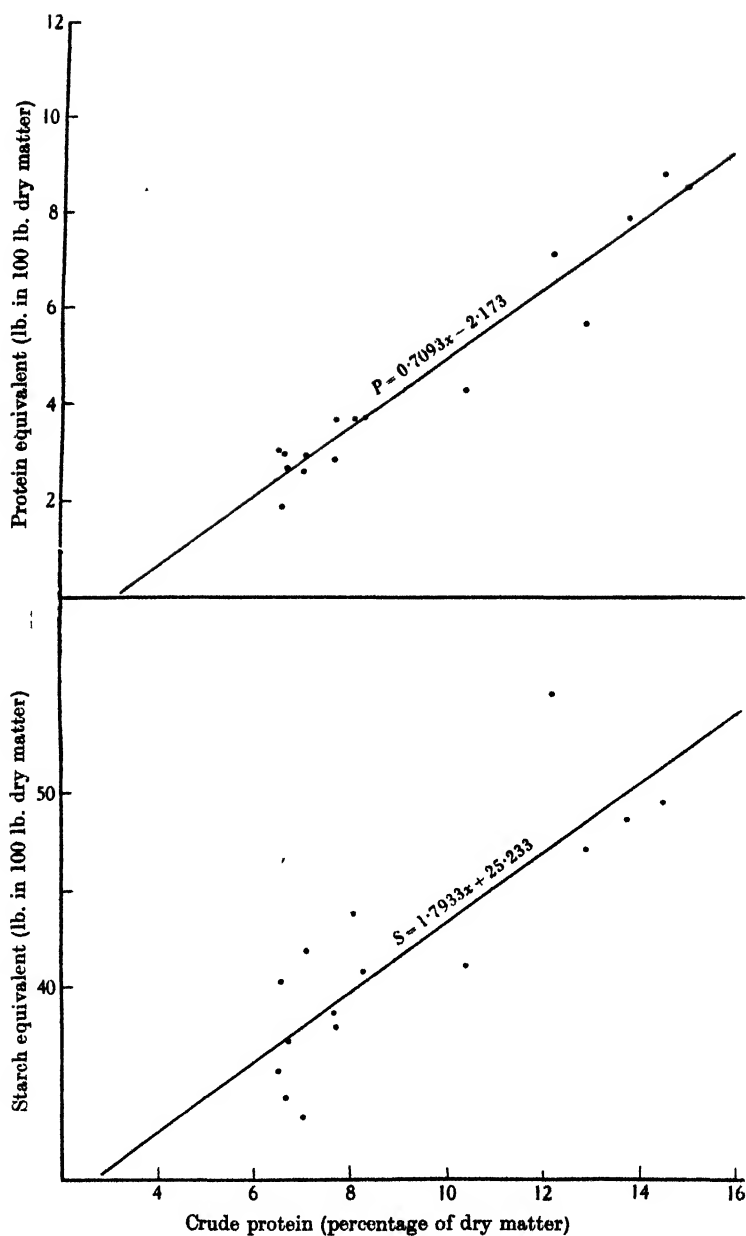


Fig. 2. Hay.

the present year on a number of hays of different type and known treatment to obtain more complete data on the nutritive value of average hay.

A curve (Fig. 2) has been prepared from the data obtained at Jealott's Hill which may serve as a means of estimating approximate starch and protein equivalent values from the crude protein content of leafy types of hay. It may, however, prove necessary to revise this in the light of further information.

### SILAGE

The samples of pit and tower silage have been grouped irrespective of the method of making adopted, since it would appear that there is little difference in composition and digestibility as between the processes where a suitable container has been used to make the silage.

The samples of silage made in pits do not refer to simple earth-lined pits, but were made in wooden or concrete containers sunk for a part or a whole of their depth in the ground and fitted with a movable over-silo usually made of wood. The average depth was 5 ft. with an over-silo of like height, the finished silage settling within the pit after which the over-silo was removed.

The silage from tower silos may be classed with the silage from pits. An examination of the figures shows that all the silages were of high digestibility with the exception of No. 1, which was made from material which was beyond the stage at which herbage should be cut for silage making. It is also noticeable that practically all the silages fall into two distinct classes, one with a protein content between 13 and 15 per cent. of the dry matter, the other 17-19 per cent. Three samples lie below 13 per cent. of crude protein and four above 19 per cent. The silages in the first category correspond to the type of silage produced from surplus grass in early summer (early June), the second and higher quality type being usually obtained in the autumn and in May from short grass. The two distinct types are summarised below. In the main tables all the starch equivalent values have been calculated by the method first advocated by Kellner(2), in which the energy value of the nitrogenous constituents is based on the digestible "true" protein. In the case of silage a corrected value has been inserted in Tables III and IV, based on the digestible crude protein. This is probably a more accurate figure, except in cases where the formation of volatile bases during the process has been excessive.

The starch equivalent values do not differ markedly, and it may be

assumed that for all pit and tower silages it is a fairly constant figure at about 53–55 per cent. of the dry matter. The protein equivalent rises with the crude protein content and is roughly proportional thereto. The differences which may arise owing to the degree of fermentation and the course of the chemical changes in the making of the silage cause considerable variation in the values for protein equivalent, which are, however, usually about half the magnitude of the figures for percentage of crude protein. A knowledge of the dry-matter content of the silage is of the greatest importance, and is probably of more value than any other figure, since the silage could usually be allocated on inspection to one or other of the two main types. The difference between 20 and 30 per cent. of dry matter—and such a variation may on occasion arise in one silo—will mean a 50 per cent. increase in feeding value with the higher dry-matter figure.

Table III. *Crude protein, starch equivalent and protein equivalent values of silage samples made in towers or pits*

% of dry matter ...	Up to 15 % of crude protein in the dry matter	Over 17 % of crude protein in the dry matter
Crude protein	13.09	18.63
Starch equivalent	54.7	53.2
Protein equivalent	6.15	8.71
Corrected starch equivalent*	59.6	60.1

\* Digestible crude protein used in calculating the energy value of the protein.

The values for the silage made from silage crop mixtures in a tower have not been included. The two samples show lower values for starch equivalent than was the case with grassland herbage, but the protein equivalent figures were only slightly lower.

*Silage made in a stack.* Owing to the lack of control of the biological processes in the stack, very large differences in nutritive value may be found in stack silage. A comparison of sample No. 8 with sample No. 12 shows the variation in digestibility of “true” protein, the former sample containing none. The silage in this stack (No. 8) reached a very high temperature, to which this result can be ascribed. The digestibility coefficient of the “true” protein in stack silage compared very unfavourably with that for pit or tower silage. As a result of the extreme respiration in a stack, which affects the most easily digestible fraction of the nitrogen-free extractives to the greatest degree, the coefficients of digestibility for this constituent are also lower in the stack than for other types of silage. This is also true of the fibre fraction. The average value for all the stack silages shows that they are inferior to tower or pit silage.

Table IV. *Average crude protein, starch equivalent and protein equivalent values of stack silage samples*

	% of dry matter
Crude protein	14.22
Starch equivalent	44.0
Protein equivalent	4.57
Corrected starch equivalent*	47.2

\* Digestible crude protein used in calculating the energy value of the protein.

Stack silage No. 12 shows the nearest approach to the pit and tower silages. This silage was built in a movable wooden casing, which was removed as soon as the material had settled. The better control of the silage-making process due to better compaction and the exclusion of excess of air in the early stages is reflected in the starch equivalent value and more particularly in the protein equivalent. The general improvement in digestibility and nutritive value in this sample is evidence of the value of a movable casing for use in the making of stack silage.

The breakdown in the silage process with its accompanying formation of non-protein nitrogen is reflected in relatively low values for protein equivalent. Modern work is tending to show that the non-protein nitrogen, unless the breakdown has gone too far, as in very poor quality silage, may have the same value as the original "true" protein from which it was formed. This has been demonstrated by Kirsch and Jantzon<sup>(3)</sup> and is tentatively accepted by Edin and co-workers<sup>(4)</sup>, who usually quote a corrected digestible "true" protein figure based on the original "true" protein in the crop prior to ensilage. A recent experiment which has just been concluded at Jealott's Hill<sup>(5)</sup> has indicated that the non-protein nitrogen in silage is of high value for milk production. Morris and Wright have also shown<sup>(6)</sup> that the nitrogen in silage has a high biological value. It would seem, therefore, that the present-day conception of the nutritive value of non-protein nitrogen in the ration will have to be modified in the case of silage, and the digestible crude protein may prove to be a more correct measure of its value.

*Stack silage from other crops.* The fodder made by the A.I.V. process from marrow-stem kale and sugar-beet tops is of interest in showing the application of the process to such material which was filled into the silo in an uncut condition.

*Potato silage.* Some trials on steamed potatoes and potato silage have given interesting results. The material for test was kindly supplied by Mr J. C. Wallace of the Lincolnshire (Holland) County Council Agricultural Institute. The steamed ensiled potatoes are a nutritious fodder

similar in feeding value to the freshly cooked potatoes. The raw potatoes ensiled with the addition of mineral acid gave a product of considerably lower feeding value, this being particularly noticeable with the protein which was of low digestibility. Raw potatoes were also ensiled without any addition, but the sheep would not eat the product, though it was palatable to pigs. Further information is needed using pigs as experimental animals before final conclusions can be drawn, since potatoes conserved, either raw or steamed, would normally be fed to pigs, but the results agree well with Continental experience(7).

#### ARTIFICIALLY DRIED FODDER

Most of the samples were obtained from grassland herbage and represent a mixture of grasses with a certain proportion of clovers, dried on a band drier operating with inlet gases at 250° C. The material varied from a low-quality product with just over 10 per cent. of crude protein up to one with just under 22 per cent. in the dry matter. The values for starch and protein equivalent have been plotted in Fig. 3 against the crude-protein content. The curves should afford a fairly accurate method of assessing the nutritive value of any sample of dried grass from a knowledge of its crude protein content. In the calculation of the starch equivalent of the dried grass the correction was made on the basis of a fodder of high fibre content (Kellner(2)). This entails a subtraction of 0.58 of starch equivalent for every 1 per cent. of crude fibre in the dried grass, although in the case of the fresh grass from which it was made the correction would only be 0.29–0.35. This correction which was suggested by Kellner(2) was proposed because the difference between the gross and the nett starch equivalent values was more closely related to the fibre content than to any other and not because it was necessarily due to the fibre itself. It would be more fair to artificially dried grass to apply the same lower correction which would be used for the fresh herbage.

The correction factor was used by Kellner for hay and straw, etc., which are mature products. The increase of the factor with fibre content assumes that high fibre content *due to maturity of growth* indicates lignification and hence a real loss in nutritive value; the high fibre content due to the artificial removal of water from young leafy herbage does not. Indeed in the preparation of the fresh grass sample for analysis, a product of similar high fibre content is obtained. Examination of the digestibility coefficients shows that the fibre in artificially dried grass is as highly

digestible as that in fresh grass of similar fibre content, based on the dry matter.

The application of the lower correction would increase the figures for starch equivalent by about 5 units. The data for starch equivalent and protein equivalent of the samples of artificially dried grass examined are presented as curves in Fig. 3. The broken line curve for starch equivalent shows the values where the lower correction has been applied, and is considered to represent more truly the nutritive value of artificially dried grass.

The samples of artificially dried clover and lucerne examined were of poor quality and need no comment. The chief feature of interest is the lower digestibility of the carbohydrate fraction and more particularly the fibre.

#### MISCELLANEOUS

Under this category are a number of materials which have been examined in connection with different experiments at this station.

*Marrow-stem kale.* The first four trials were carried out in connection with an experiment on the manuring of marrow-stem kale, and the results do not differ appreciably. Sample 5 was of marrow-stem kale in the early stages of growth when the crop is extremely leafy. This sample was tested in early October. The crude protein and protein equivalent contents are outstanding, but the yield at this stage would not be high. As the crop grows and the ratio of stem to leaf increases, the crop would approach the values of the first four samples.

*Sugar-beet tops and leaves.* Only one sample was examined and the results agree with those usually quoted in the literature.

*Legumes husks.* Digestibility trials on pea, bean and lentil shells, or husks, show that, with the exception of the lentil, the protein equivalent values are low though the starch equivalent values are fairly high. This work has been reported in detail elsewhere(8).

*Cereal and root crops.* The results for these trials give values which do not differ appreciably from those in the literature, although the protein equivalent of the cereals is lower than would be expected.

*Dreg meal.* This material, which is variously called dreg meal, slummage or distillery waste, was examined and the results have been reported in detail elsewhere(9).

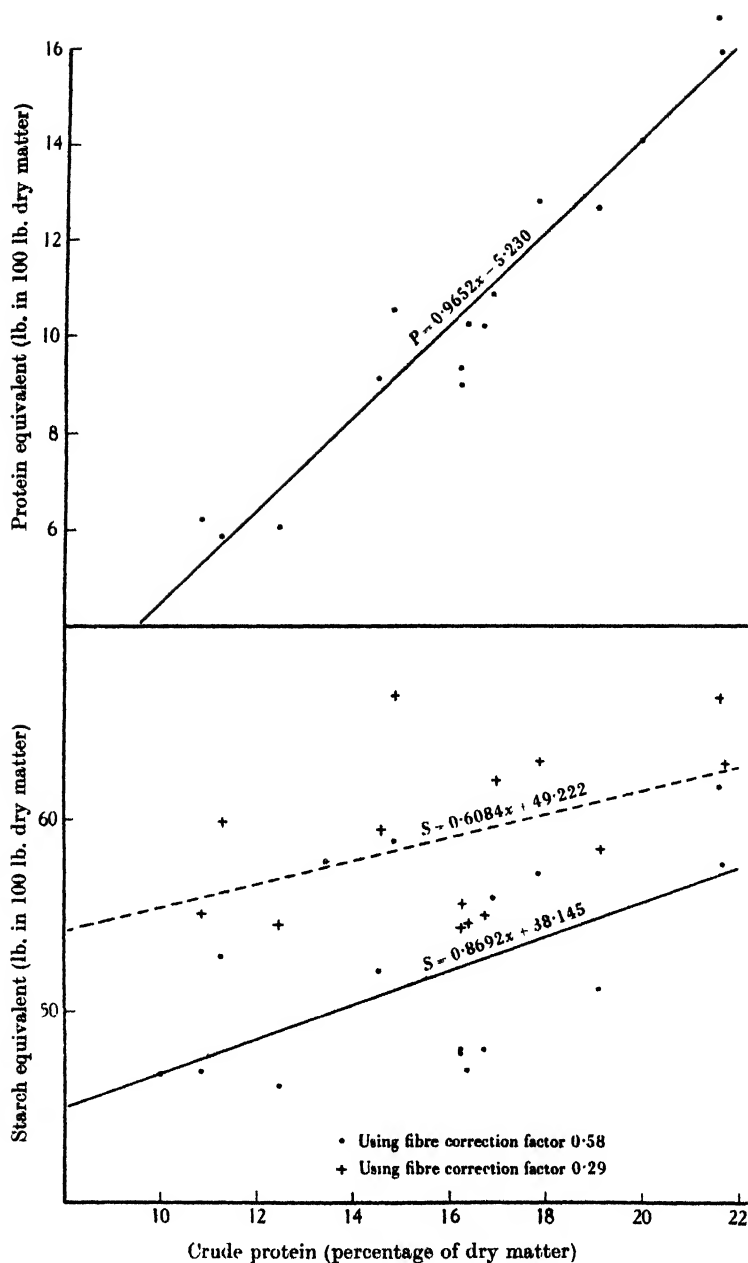


Fig. 3. Dried grass.

COMMENTS ON STARCH EQUIVALENT AND PROTEIN  
EQUIVALENT CURVES

Linear regression equations were calculated for starch equivalent ( $S$ ) and protein equivalent ( $P$ ) on crude protein ( $x$ ) for each of the three groups—dried grass, fresh grass and hay. There was no evidence that a straight line was not an adequate fit in each case for the range considered, with one exception, that the point at the highest end of the range for fresh grass was omitted, since it suggested a bend in the true curve at that level.

Owing to the high variability for the starch equivalent figures, the corresponding equations will have a high error of estimation. The protein equivalent equations are more accurately determined.

Table V. *Summary of examination of data used in the  
preparation of the curves*

	Mean	S.E.* of mean	Regression coefficient $b$	S.E.* of $b$	Regression equation
Starch equivalent:					
Dried grass	59.076	1.091	+0.6084	0.3426	$S = 0.6084x + 49.222$
Dried grass†	52.223	1.196	+0.8692	0.3756	$S = 0.8692x + 38.145$
Fresh grass	57.680	0.953	+0.6886	0.2225	$S = 0.6886x + 47.971$
Hay	41.547	0.904	+1.7933	0.3270	$S = 1.7933x + 25.233$
Protein equivalent:					
Dried grass	10.402	0.243	+0.9652	0.0764	$P = 0.9652x - 5.230$
Fresh grass	9.176	0.131	+0.8545	0.0306	$P = 0.8545x - 2.872$
Hay	4.280	0.158	+0.7093	0.0572	$P = 0.7093x - 2.173$

\* For values of  $x$  obtained.

† Calculated with fibre correction factor 0.58.

The values obtained for silage did not appear sufficiently homogeneous for it to be worth while calculating regression equations for this group, even after these values had been separated into two sub-groups, silage with acid and without acid.

### SUMMARY

The composition, digestibility coefficients and digestible nutrients of a large number of samples of fresh grass, silage, hay and artificially dried grass are tabulated. Curves have been prepared from these figures which enable the starch equivalent and protein equivalent to be obtained, by interpolation, from the value for crude protein determined by analysis.

The method of calculating the starch equivalent of artificially dried grass from the data for digestibility and composition is discussed with



particular reference to the fibre correction factor. It is considered that this should be the same as that used for the fresh grass prior to drying.

A number of miscellaneous samples—mostly of home-grown foods—are included in the tables.

#### ACKNOWLEDGMENTS

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# EXPERIMENTS ON VERNALISATION

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(With Twenty-six Text-figures and One Graph)

## INTRODUCTION

PRELIMINARY experiments have demonstrated that when the soaked grain of some varieties of wheat, barley and oats are exposed to low temperatures the subsequent growth of the plants may be markedly affected(1). There was evidence to suggest that the low-temperature treatment stimulated the early growth rate in all the varieties under observation, while the juvenile habit in some varieties assumed a greater erectness. Further evidence for a complete physiological stimulation by the low-temperature exposure was seen in a greater luxuriance of growth, an increased rate of growing point development, and an acceleration in earing. Winter varieties were more responsive to all these phases of stimulation than were the spring varieties, but the results of the observations throughout the growing season strongly suggested that the effectiveness of the low-temperature treatment was dependent on the subsequent growing conditions.

The first two experiments described in this paper were designed to investigate further two aspects of this preliminary work. First, by making detailed observations of treated and control plants when sown at different times, the effect of the growing conditions on varietal responsiveness could be studied. Secondly, if early growth rate is stimulated by low-temperature treatment this should be discernible by measuring the rate of tillering of treated and control plants.

## I. THE EFFECT OF TIME OF SOWING ON VERNALISATION RESPONSE

Although the main object of the experiment was to determine whether the effect of vernalisation treatment was dependent on the light and temperature conditions during subsequent growth, the plants were also used for comparative studies on low-temperature stimulation at different phases of growth. Varietal behaviour will therefore be discussed in conjunction with the main problem.

Seven separate sowings of vernalised and control plants were made

during the autumn and spring of 1934-5. The single autumn sowing was made on October 26, while the first spring sowings were made on March 8. Of these spring sowings, one was made in the field, while the other was made in the glasshouse, the plants being protected from low-temperature exposure for the first 4 weeks of growth. Four more spring sowings were made in the field at 7-day intervals on March 15, March 22, March 29, and April 5. The low-temperature treatment in all cases consisted of exposure for 14 days to a temperature of 3° C.

*Germination acceleration.* With the exception of the winter sowing of the variety Wintergerste,<sup>1</sup> all vernalised sowings showed germination acceleration (Table I). The amount of acceleration was not as great as in the preliminary investigations of the previous year, a fact which may be partially explained by the better control of the vernalised grain during treatment. Nevertheless, observations on material sown in soil, whether in pots or in the field, can scarcely be sufficiently accurate to demonstrate adequately differences in the rate of germination. Probably the only method of studying germination acceleration is by controlled laboratory observations by which actual measurements of coleoptile elongation can be made. Even with careful work of this description, there will have to be large differences in growth rate to show with any certainty that stimulation has taken place.

Table I. *Germination acceleration in days*

	Oct. 26 Field	Mar. 8 Field	Mar. 8 Glass- house	Mar. 15 Field	Mar. 22 Field	Mar. 29 Field	Apr. 5 Field
<i>Hordeum</i>	2	—	—	—	—	—	—
<i>spontaneum</i>							
Pryor's	—	1	2	2	2	2	1
Spratt-Archer	—	1	2	2	3	2	1
Tschermak's	—	2	1	2	2	2	1
Wintergerste	0	2	2	3	2	2	1
Bocumer	1	2	2	3	3	2	1
Yeoman	2	1	1	2	2	2	1
Joss	2	1	2	2	2	3	2
Rivett	2	2	2	2	2	2	1
Grey Winter	4	2	2	3	3	2	1
Black Winter	—	2	1	3	5	2	2
Abundance	—	3	2	3	3	2	2

The only suggestion that the time of sowing affects the germination acceleration is to be seen in the latest spring sowing, where the differences between control and vernalised plants are reduced to a minimum in most varieties. There is no evidence to suggest that the degree of acceleration is associated with the period of germination.

<sup>1</sup> Stadler's. A two-row winter barley.

*Juvenile habit.* The observations on all sowings show that the juvenile habit and general appearance of the young plants may be entirely altered by vernalisation treatment. This juvenile effect is a varietal character, however, and is also affected by the time of sowing. The barley variety, Tschermak's, was the most responsive, the vernalised plants being semi-erect and nearly twice as large as the small, prostrate, untreated plants. The other winter barleys, Bocumer and Wintergerste, also showed large treatment effects, but they were not as obvious as those of Tschermak's. Rivett responded most obviously of the wheats, while Black Winter and Grey Winter oats were more responsive than Abundance oats, which, with Spratt-Archer and Pryor's barleys, showed little more than a small increase in luxuriance.

The effect of the time of sowing is simple but interesting. In the October sowing there was little difference between the treated and untreated plants because the low field temperature prevented rapid growth and induced procumbency in the vernalised plants, thereby causing them to resemble the controls. In the spring sowings the greatest treatment effects were apparent in the earliest sowing, and became less as the sowing time became later. This may be explained by the fact that the habit of the control plants became more erect, and thus resembled the vernalised plants more closely, with increased lateness of sowing.

*Growing point development.* The growing points of cereals afford a reliable index of the plant's growth and passage from the vegetative to the reproductive phase. Consequently, by studying the relative growth rates of the growing points, an accurate reflection of the development of the plant can be obtained. It was, therefore, decided to examine the growing points of vernalised and untreated plants in order to discover when vernalisation stimulation was first apparent in the different varieties, and also to trace differences in the rates of development of these growing points.

It was not possible to study the plants from one sowing only, because the sacrifice of plants would have prevented accurate observations on the later phases of plant development. Therefore, plants were taken from different sowings and examined microscopically on the same day as the samples were taken. In this way it was possible to compare the growing points of the different varieties at various ages and so to obtain some idea of varietal behaviour. Camera lucida drawings were made from the freshly dissected growing points, and these are represented in the accompanying figures.

In each set of figures A signifies the control and B the treated plant.

Some twelve plants were examined in each case before a representative growing point was chosen as typical of the phase of development of the whole sample. The examination was confined to the main tiller of each plant, and a surprising uniformity between the growing points of the plants of any one sample was apparent.

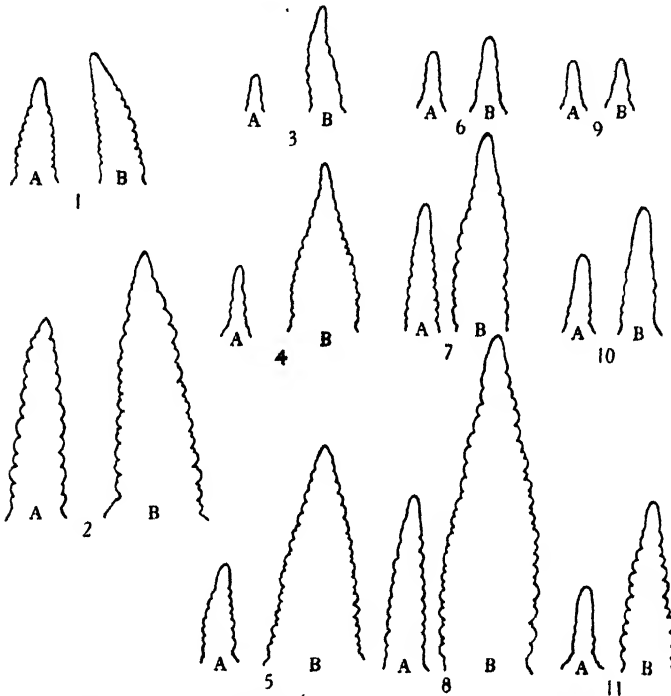


Fig. 1. Spratt-Archer, 35 days.

Fig. 2. Pryor's, 35 days.

Fig. 3. Tschermak's, 34 days.

Fig. 4. Tschermak's, 45 days.

Fig. 5. Tschermak's, 52 days.

Fig. 6. Wintergerste, 34 days.

Fig. 7. Wintergerste, 48 days.

Fig. 8. Wintergerste, 59 days.

Fig. 9. Bocumer, 34 days.

Fig. 10. Bocumer, 48 days.

Fig. 11. Bocumer, 59 days.

Examination of the figures shows that the spring barleys, Spratt-Archer and Pryor's, have the most highly developed growing points at the youngest stages, but they show only a small vernalisation stimulation at any time. Perhaps if earlier examinations had been made more obvious differences between the control and treated plants would have been found. Pryor's shows a greater stimulation than Spratt-Archer (Figs. 1 and 2).

The winter barleys, Tschermak's, Wintergerste and Bocumer, are characterised by very definite growing point stimulation. Tschermak's

is the only variety which shows obvious differences at the youngest stage (34 days), and the relatively greater rate of development of the vernalised growing points is apparent at the later stages (Figs. 3-5). Wintergerste is somewhat slower to show the vernalisation effect, and there is never the great difference between the vernalised and control growing points, because the controls grow more quickly in relation to the vernalised plants than they do in Tschermak's (Figs. 6-8). Bocumer

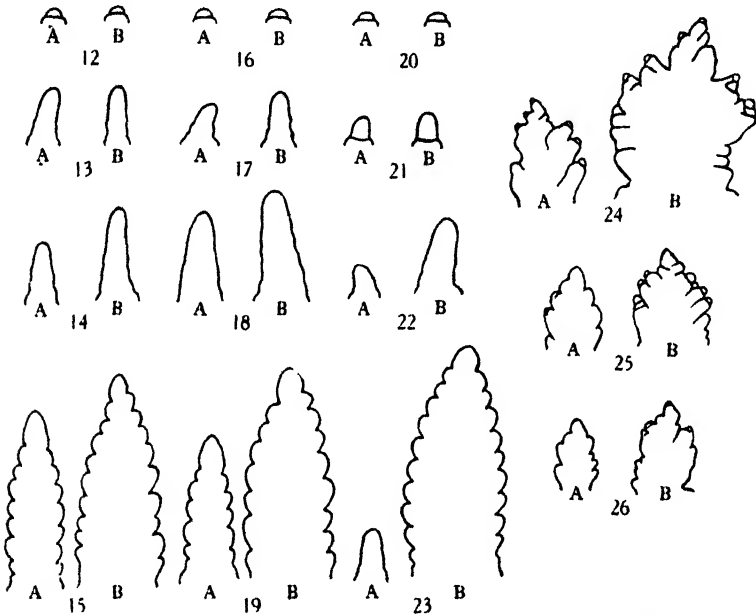


Fig. 12. Yeoman, 34 days.  
 Fig. 13. Yeoman, 52 days.  
 Fig. 14. Yeoman, 69 days.  
 Fig. 15. Yeoman, 77 days.  
 Fig. 16. Joss, 34 days.  
 Fig. 17. Joss, 52 days.  
 Fig. 18. Joss, 69 days.  
 Fig. 19. Joss, 77 days.

Fig. 20. Rivett, 34 days.  
 Fig. 21. Rivett, 52 days.  
 Fig. 22. Rivett, 69 days.  
 Fig. 23. Rivett, 77 days.  
 Fig. 24. Abundance, 67 days.  
 Fig. 25. Black Winter, 67 days.  
 Fig. 26. Grey Winter, 67 days.

is the slowest of the three winter barleys in growing point development, and it is not until the 59-day stage that the vernalised growing points grow away from the controls (Figs. 9-11).

The winter wheats are characterised by a much slower rate of growing point development than the winter barleys, and examination of the growing points of Joss, Yeoman and Rivett at 34 days and 52 days show them to be small and completely undifferentiated (Figs. 12 and 13, 16 and 17, 20 and 21). At 61 days, differences in the rate of development



of the three varieties can be seen, with Rivett the slowest, but again there are no signs of vernalisation effect. It is not until 69 days that the vernalised growing points exhibit a greater growth rate, which is most apparent in Rivett although the rate of growth is slower than in the other two varieties (Fig. 22). At this stage Joss appears the most advanced of the three wheats, but it shows the least vernalisation effect (Fig. 18). At 77 days Rivett is remarkable for the rapid development of the vernalised growing points, while the control has made very little growth (Fig. 23). This is in marked contrast to Joss and Yeoman, where the control growing points have developed much more rapidly, so that there are comparatively small differences between them and those of the treated plants (Figs. 15 and 19).

The oat varieties showed no vernalisation effect until roughly the same time as the wheat varieties. Figs. 24-6 show the three varieties at 67 days, which are comparable with the wheat figures at 69 days because they are from the same sowing date. Abundance is the furthest advanced and shows the greatest vernalisation effect, while Grey Winter is the slowest to develop, but manifests a marked stimulation in the treated growing point. Black Winter is intermediate in growth phase, and the vernalisation effect is the least apparent of the oat varieties.

*Earing acceleration.* Growing conditions must affect the degree of earing acceleration afforded by any vernalisation treatment, just as they affect the responsiveness of treated plants during the earlier phases of growth. The most obvious environmental factor affecting earing acceleration is the temperature during early growth, because exposure of the control plants to low temperatures during and after germination will stimulate them to ear earlier than if the early temperature conditions were high. Evidence in support of this view was obtained in the preliminary experiments where sowing on the same date in the field and in the glasshouse resulted in greater earing acceleration in the glasshouse because of the later earing of the control plants in the glasshouse than in the field<sup>(1)</sup>.

If vernalisation brings about the same result as autumn sowing, there should be little difference between the earing time of control and vernalised plants when sown in the autumn, provided the vernalisation treatment exposes the plants to the necessary low-temperature requirement. Naturally it is very unlikely that the vernalisation treatment will coincide with the field low-temperature exposure through the winter, so that small differences are inevitable. With regard to spring sowings, it is to be expected that the degree of earing stimulation brought about

by any vernalisation treatment will become more pronounced with increased lateness of sowing, until the temperature and light conditions are such as to prevent the vernalised plants from coming into ear.

Varietal behaviour in the seven sowings supports the views already expressed (Table II). Autumn sowing prevents any earing acceleration in five out of the seven varieties, while in the other varieties there is a small acceleration due to vernalisation. In the spring sowings the general trend for the field-sown plants is a progressive increase in earing acceleration for the winter varieties, and a progressive decrease for the spring varieties as the sowing becomes later. There are, however, exceptions to this general trend in the intermediate sowings of March 22 and March 29 which are difficult to explain. Strong evidence in favour of the effect of low temperature during early growth on vernalisation effectiveness in accelerating earing is supplied by a comparison of the March 8 sowings in the field and in the glasshouse. The glasshouse protection in every case meant a much greater difference in the earing times of the control and vernalised plants of the winter varieties largely because the control plants were much later coming into ear in the glasshouse than in the field.

Table II. *Earing acceleration in days*

	Oct. 26 Field	Mar. 8 Field	Mar. 8 Glass- house	Mar. 15 Field	Mar. 22 Field	Mar. 29 Field	Apr. 5 Field
<i>Hordeum spontaneum</i>	1	—	—	—	—	—	—
Pryor's	—	1	3	1	1	0	0
Spratt-Archer	—	1	3	2	1	1	1
Tschermak's	—	4	22	8	8	6	13
Wintergerste	0	2	24	12	4	4	20
Bocumer	2	3	20	9	10	10	17
Yeoman	0	0	10	3	0	2	7
Joss	0	2	11	3	7	12	12
Rivett	0	3	15	9	13	11	17
Grey Winter	0	1	3	3	3	2	7
Black Winter	—	2	3	1	1	1	4
Abundance	—	0	1	0	2	0	0

A more accurate picture of the behaviour of each variety is given by Table III, which shows the number of days from sowing to earing in each variety for each of the sowings. The autumn sowing requires no discussion because the number of days to earing is obviously a reflection of the comparative cessation of growth through the winter. Comparison of the two March 8 sowings, however, illustrates the difference in the behaviour of winter and spring varieties.

The spring varieties—Pryor's, Spratt-Archer and Abundance—are

scarcely affected by vernalisation as far as earing is concerned and the number of days from sowing to earing is largely conditioned by the direct effect of temperature on growth rate. Thus, as the temperature during early growth becomes higher, the growth rate increases and the number of days to earing decreases in both vernalised and untreated plants. This is best seen by comparing the glasshouse and field sowings of March 8 in which the period to earing is considerably shortened by growing in the glasshouse during the first few weeks. There is also a progressive shortening of the time necessary to come into ear in both control and vernalised plants as the sowing becomes later.

Table III. *Number of days from sowing to ear emergence*

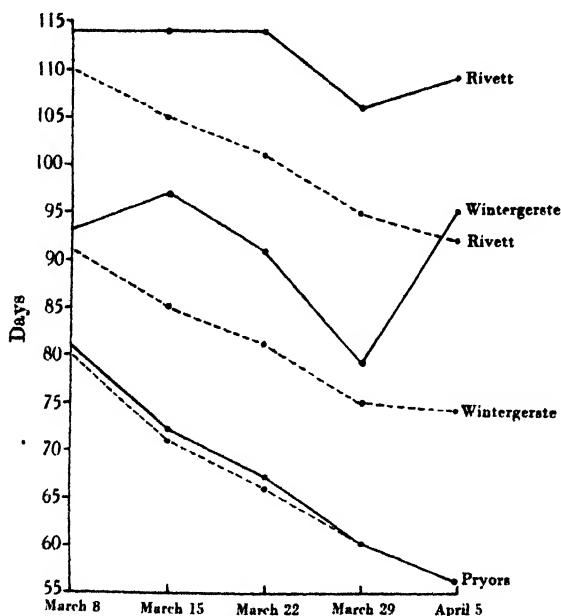
		Oct. 26	Mar. 8	Mar. 8	Mar. 15	Mar. 22	Mar. 29	Apr. 5
		Field	Field	Glass-house	Field	Field	Field	Field
<i>Hordeum</i>	A	194	—	—	—	—	—	—
<i>spontaneum</i>	B	193	—	—	—	—	—	—
Pryor's	A	—	81	63	72	67	60	56
	B	—	80	60	71	66	60	56
Spratt-Archer	A	—	101	95	94	86	84	77
	B	—	100	92	92	85	83	76
Tschermak's	A	—	92	108	89	84	79	81
	B	—	88	86	81	76	73	68
Wintergerste	A	208	93	119	97	91	79	95
	B	208	91	95	85	81	75	74
Bocumer	A	214	102	116	101	97	93	100
	B	212	99	96	92	87	83	83
Yeoman II	A	230	113	124	107	98	95	96
	B	230	113	114	104	98	93	89
Joss IV	A	233	113	128	108	105	106	102
	B	233	111	117	105	98	94	90
Rivett	A	233	114	129	114	114	106	109
	B	233	111	114	105	101	95	92
Grey Winter	A	227	102	105	98	92	86	84
	B	227	101	102	95	89	84	77
Black Winter	A	—	104	105	99	90	86	82
	B	—	102	102	98	89	85	78
Abundance	A	—	103	100	97	91	85	79
	B	—	103	99	97	89	85	79

A = control. B = vernalised.

Winter varieties do not behave in this manner, because the control plants require a longer period to come into ear when sown in the glasshouse than when sown in the field on the same date. This is true of all varieties tested with the exception of the three spring varieties mentioned above. The vernalised plants of these winter varieties are affected differently by the two sowings. Tschermak's and Bocumer were characterised by a reduction in the period to earing when sown in the glasshouse, indicating that the vernalisation treatment was more effective than the field temperatures in stimulating earing. Yeoman, Grey Winter and Black Winter were comparatively unaffected by the two treatments,

although the tendency in the former two was for a longer period to earing in the glasshouse. Wintergerste, Joss and Rivett were able to reduce the period to earing further in the field sowing than in the glasshouse sowing, suggesting that field vernalisation was more effective than the vernalising effect of the low-temperature chambers.

The behaviour of the control and vernalised plants of the winter wheat and winter barley varieties for the five spring sowings illustrates the "vernalising effect" particularly well. In all these varieties—



Graph 1. Number of days from sowing to ear emergence of control (—) and vernalised (---) plants.

Tschermak's, Wintergerste, Bocumer, Joss, Yeoman and Rivett—the control plants tend to show a progressive shortening of the period to earing from the March 8 sowing to the March 29 sowing. There are, however, exceptions to this, as, for example, in some of the sowings of Wintergerste, Joss and Rivett. In the last sowing the number of days to earing increases compared with the penultimate one in all varieties except Joss. For the most part this increase is not large, but it should be emphasised that the earing of the control plants of all winter varieties in the last sowing was very uneven and spread over a long period of time, and even after 2 or 3 weeks all the plants had not come into ear.

It was therefore necessary to take the earing of the first plant as the earing date of the whole plot. This means, of course, that the figures stated underestimate the true state of affairs, and in many ways it would be more accurate to state that the control plants failed to ear normally. When the treated plants of these varieties are compared with their controls it may be seen that with the exception of the last sowing of Bocumer there is a steady reduction in the number of days to earing as the sowing becomes later, *i.e.* the treated plants are able to behave like spring varieties (Graph 1). Grey Winter and Black Winter oats behave like spring varieties in the progressive shortening of the period to earing of the control plants, but they are also characterised by an earing acceleration which places them between the spring and winter varieties.

The behaviour of the winter varieties in this experiment supports the view already put forward by other investigators that long days retard ear formation in winter wheats, but long days accelerate ear formation when the grain has been previously vernalised(2). This hypothesis apparently holds for winter barley as well, because the behaviour of the winter barley varieties in the present study was identical with that of the wheats. It should be emphasised that none of the varieties used in this work is a true winter variety, but each possesses some characters of the spring varieties. This is particularly true of the winter oat varieties, Grey Winter and Black Winter, whose behaviour can only entitle them to be called "semi-winter".

### *Conclusion*

To conclude this account of the effect of the time of sowing on the responsiveness to vernalisation treatment it is interesting to correlate the behaviour of the different varieties at the various stages of development. The winter barleys—Tscherma's, Wintergerste and Bocumer—were subject to the most marked vernalisation effects at all growth phases; each showing large juvenile effects, strong growing point stimulation, and the greatest acceleration in earing. Tscherma's was undoubtedly subject to the greatest juvenile stimulation, and at this period in its development it also exhibited the largest growing point stimulation. Although growing point stimulation is very marked in each of these three varieties it is first apparent at different stages, and the later stimulated variety Bocumer shows as great an acceleration in earing as the earlier stimulated variety Tscherma's. Earliness of growing point stimulation is therefore not necessarily a criterion of the degree of earing acceleration. This is very well illustrated by considering the wheat

varieties, which do not show any growing point stimulation until a considerably later stage of growth than do the winter barley varieties. Each of the wheat varieties shows growing point stimulation at about the same stage of development, but Rivett eventually is subject to a much greater earing acceleration than either Joss or Rivett.

It is therefore only by making comparative studies of the growing points at various stages of development that the true degree of stimulation can be gauged. Rivett is characterised by a sudden quick development of the vernalised growing points, while the controls remain comparatively undeveloped. Joss and Yeoman, on the other hand, do not reveal this great growing point difference because the control plants are subject to a greater relative rate of development. It is worth while noting that these growing point differences are reflected in the juvenile habit changes which are most apparent in Rivett.

The least responsive varieties at all stages of growth are the oats and the spring barleys. The spring barleys are characterised by very rapid growing point development with but a small vernalisation stimulation. There is little doubt, however, that Pryor's is affected more obviously in the early stages than the difference in earing acceleration would suggest. The oat varieties show a much slower rate of growing point development, with a somewhat greater vernalisation stimulation. Again, there is little doubt that the greater earing acceleration of Grey Winter and Black Winter is reflected in the more obvious changes in juvenile habit, and more detailed growing point examination at later stages would probably reveal more obvious vernalisation stimulation.

These comparative developmental studies have revealed the essential stimulative action of low-temperature treatment, and they have also shown how winter and spring varieties may be distinguished by studying the growing point development. In the winter varieties the low temperature stimulates the earlier differentiation of the ear primordia, thus hastening the passage from the vegetative to the reproductive phase. This stimulation may be comparatively late in manifesting itself, but once it is apparent the greater rate of development of the growing points that have been exposed to low temperatures is quite obvious. Although some stimulative action may be seen in spring varieties there is never the great difference in the relative rates of development of the vernalised and control growing points. It is therefore probable that low-temperature treatment may offer a means of distinguishing spring and winter forms by examination of the growing points, a fact which may have application in the isolation of desirable strains in plant breeding.

## II. THE EFFECT OF VERNALISATION TREATMENT ON THE RATE OF TILLERING

The rate of tillering of cereals is an important character for measuring the rate of growth during the vegetative phase, while the number of tillers produced is a guide to the vegetative luxuriance and partially determines the number of ears at harvest. If low-temperature treatment stimulates early growth rate and hastens the passage from the vegetative to the reproductive phase, there should be a reflection of this in an increased tiller rate of treated plants, when compared with the control plants of the same variety.

In order to investigate differences in the rate of tillering due to vernalisation an experiment was planned on the basis of a simple randomised Latin square. The wheat varieties Yeoman, Joss and Rivett were vernalised for 14 days at 3° C., and with the controls of each variety were replicated six times each in a thirty-six plot trial. One hundred and twenty grains were sown per plot, making seven hundred and twenty grains for each vernalised and control variety. Two rows of untreated wheat surrounded the whole trial in order to eliminate as far as possible any edge effect. Unfortunately, the sowing, which was made on March 16, coincided with dry conditions, and there was an abnormally great loss of plants during germination. The plants were counted in each plot, however, and the resulting figures, although showing that the Joss plots had suffered most severely, gave no reason to suppose that there was any difference in the survival rate of the control and treated plants of any variety.

The first tiller count was made, on April 15 when tillering had commenced in all plots, although the majority of plants did not possess tillers. The difference in the number of tillers of the control and vernalised plants was so obvious that no statistical analysis is necessary to demonstrate that the vernalisation treatment had stimulated tillering in each variety (Table IV). It is also obvious that Rivett, although the slowest tillering variety, is subject to the greatest stimulation, while Yeoman is stimulated to a greater degree than Joss, although the rate of tillering in the control plants is identical in these two varieties.

Table IV. *Count 1. Total tiller number for the six plots of each control and vernalised variety*

	Yeoman	Joss	Rivett
Control	102	101	15
Vernalised	257	208	104

Subsequent tiller counts were made on April 25, May 16, and on August 8, when the plants were<sup>17</sup> mature. In each of these counts the data were subjected to the analysis of variance, and significant differences were based on Fisher's  $Z$  test. In the brief account which follows the figures given are restricted to the minimum commensurate with a proper substantiation of the conclusions drawn from this experiment.

Table V. *Count 2. Average tiller number of six plots of control and vernalised plants of each variety*

	Yeoman	Joss	Rivett	Mean	
Control	1.38	1.28	0.88	1.18	S.E. = 0.2667
Vernalised	1.67	1.49	0.92	1.36	
Mean	1.53	1.39	0.90		

From this tiller count it may be concluded that the rate of tillering is significantly higher ( $Z=1.958$  as against 1.045 for  $P=0.01$ ) in the vernalised plots than in the control plots. The vernalisation effect is, however, a varietal character, being greatest in Yeoman, less in Joss, and smallest in Rivett. This is substantiated by the interaction effect being significant at the 5 per cent. point ( $Z=0.705$  as against 0.6254). It is also worth noting that the rate of tillering in each variety is individualistic, showing significance at the 1 per cent. point ( $Z=1.1866$  as against 0.8831).

Table VI. *Count 3. Average tiller number of six plots of control and vernalised plants of each variety*

	Yeoman	Joss	Rivett	Mean	
Control	8.41	9.08	8.01	8.50	S.E. = 1.856
Vernalised	7.24	6.66	5.83	6.58	
Mean	7.83	7.87	6.92		

This tiller count shows clearly that the rate of tillering in the control plants is higher than the rate in the vernalised plants ( $Z=2.0349$  compared with 1.045 for  $P=0.01$ ). Further, the inhibitive action of the vernalisation treatment is more marked in Joss and Rivett than in

Table VII. *Count 4. Average tiller number of six plots of control and vernalised plants of each variety*

	Yeoman	Joss	Rivett	Mean	
Control	1.43	1.92	1.34	1.59	S.E. = 0.4367
Vernalised	1.42	1.73	1.16	1.44	
Mean	1.425	1.825	1.25		



Yeoman ( $Z$  for interaction = 0.82508 compared with 0.6254 for  $P=0.05$ ). At this stage there is no significant varietal effect on the rate of tillering.

The final tiller count shows that the inhibition of tiller production by vernalisation treatment is reflected by the smaller number of ear-bearing tillers surviving at harvest in the vernalised plants ( $Z=0.7567$  as against 0.7352 for  $P=0.05$ ). Nevertheless, the significance of the interaction ( $Z=0.7912$  as against 0.6254 for  $P=0.05$ ) shows that Yeoman is not subject to any reduction in ear number by the treatment. The three varieties differ in their ear survival, Joss having the greatest and Rivett the smallest ( $Z=2.157$  as against 0.8831 for  $P=0.01$ ), but Rivett is subject to a greater relative reduction in ears surviving to harvest than is Joss.

From these observations on tillering there seems little doubt that low-temperature treatment has a definite effect on the tillering rate in Yeoman, Joss and Rivett. In the earlier stages of growth the vernalised plants tillered more rapidly than the control plants, but in the later stages the control plants possessed more tillers than the vernalised plants. Finally, at harvest, Joss and Rivett showed a greater survival of ears in the control plants as compared with the vernalised.

The obvious interpretation of these facts is that the stimulation to early growth rate leads to an increase in the tillering rate, but owing to the shortening of the vegetative phase of the plants, the total number of tillers produced is less in the vernalised plants than in the controls. This may, or may not, be reflected in a smaller number of ears surviving at harvest. Unfortunately a severe attack of "take-all" prevented any comparison of yield of grain, but this will be the object of a future investigation. Unless the earlier tillering, accompanied by the earlier formation of ear primordia, results in the production of larger ears with a greater number of superior grain, it is probable that vernalisation treatment will lead to a reduction in yield.

### III. THE EFFECT OF DIFFERENT VERNALISATION TREATMENTS ON YEOMAN AND RIVETT

Yeoman and Rivett were subjected to eight different vernalisation treatments, viz. exposure for 7, 14, 21 and 28 days at temperatures of 3 and 1° C., and were sown with controls on March 22. By the beginning of June there were differences in juvenile habit between the different treatments. Each treatment had the same effect on both varieties, but Rivett showed the treatment differences more clearly. In both varieties

the control plants were the smallest and most procumbent, and the plants were larger and more erect as the exposures became longer at both temperatures. There appeared to be little difference between the effect of the different temperatures for the shorter lengths of exposure, but for the 21- and 28-day exposures 1° C. was more effective than 3° C.

The earing acceleration was as follows:

		7 days	14 days	21 days	28 days
Yeoman	1° C.	1	1	4	4
	3° C.	1	2	3	3
Rivett	1° C.	6	7	9	10
	3° C.	7	8	9	9

Rivett showed greater earing acceleration than Yeoman at each temperature, a fact which follows naturally from the greater responsiveness of Rivett when sown at different times of the year. The effect of the different treatments suggests that 3° C. is more effective at the shorter exposures of 7 and 14 days, while for the longer exposures 1° C. is more effective than 3° C. Apparently there is little benefit in extending the exposure from 21 to 28 days, the greatest effect being obtained from increasing the exposure from 14 to 21 days.

### *General conclusions*

The most significant results of these experiments are in relation to the effect of the time of sowing on vernalisation response, and the influence of vernalisation treatment on the rate of tillering.

The time of sowing experiment showed clearly that the growing conditions control the responsiveness of treated plants, the environmental effect being most marked in the winter varieties. There appears to be little doubt that the temperature conditions during early growth constitute the most important factor of the environment influencing the efficacy of any low-temperature treatment.

The effect of the low-temperature treatment on the tillering rate of the wheat varieties emphasises very strongly how acceleration in earing is reflected in a curtailing of the vegetative growth. Although the growth rate was increased by vernalisation, the shortening of the vegetative phase prevented the normal tiller development. Whether this is reflected in the final yield remains to be seen, but there is definite evidence that the number of surviving ears may be reduced.

Vernalisation pretreatment has claimed the attention of agriculturists largely because of its effect in accelerating the onset of the reproductive

phase. In the case of cereals this has made possible the successful cultivation of wheat in areas where cultivation had hitherto been a hazardous task. In the British Isles it is doubtful whether there are any cereal-growing areas which would benefit by the application of vernalisation treatment, if it only led to a slight acceleration in the time of earing. Unless the vernalisation of spring-sown winter varieties gives better results than the cultivation of spring varieties, there is no practical utility at present for such crops.

The general results of these experiments does point to other possibilities, however. The low-temperature treatment led to a stimulation and modification of growth at all phases of development. There are vegetable crops of a perishable nature which can only be marketed over restricted periods of time because of the limited range of maturation periods of the varieties. Vernalisation may serve to protract this restricted period, and in crops which are grown for vegetative parts quick early growth may be of immense advantage even though the weight of produce suffers in consequence. It seems desirable that attention should be paid to aspects of vernalisation stimulation other than acceleration in earing, and that crops other than cereals should be utilised in experimental work.

### SUMMARY

1. The effect of low-temperature treatment on some varieties of wheat, barley and oats has been found to be profoundly affected by the time of sowing of the treated grain.
2. Winter sowing showed the least vernalisation effect, while in the spring the stimulative action became more pronounced as far as earing acceleration is concerned, as the sowing was made progressively later. This was particularly marked in winter varieties.
3. Developmental studies of the control and vernalised plants showed the stimulative action on early growth and growing point development in the winter varieties.
4. Tiller counts on control and vernalised plants of three winter wheat varieties demonstrated the fact that each variety was stimulated to earlier tiller production, but in two of the varieties this resulted in a reduction of surviving ears at harvest.
5. Six different vernalisation treatments practised on two winter wheat varieties suggested that for shorter exposure of 7 or 14 days, 3° C. was more effective than 1° C., which gave greater stimulative action at the longer exposures of 21 and 28 days.

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## REDUCTION OF NODULE NUMBERS AND GROWTH, PRODUCED BY THE ADDITION OF SODIUM NITRATE TO LUCERNE IN SAND CULTURE

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Station, Harpenden*)

(With Five Text-figures)

It is well known that the presence of nitrate in the root environment tends to reduce nitrogen fixation in nodule-bearing species of leguminous plants. A careful quantitative study of this effect in the case of lucerne (*Medicago sativa*) and soy beans (*Glycine hispida*) has been made by Giöbel (1926). A large number of papers have been published recording the effect of nitrates and ammonium salts in reducing the number of nodules produced on legumes, and nodulation has been observed to be prevented at high concentrations of these nitrogenous salts. Literature on these subjects is quoted by Giöbel (1926) and by Fred, Baldwin, and McCoy (1932), and need not be given here *in extenso*.

It is difficult to compare the results obtained by different workers, partly because of the varied methods of expressing the amounts of nitrogen compounds applied, and because of the often insufficient recording of experimental details. Part of the difficulty is also due to the fact—sometimes too little appreciated—that after a dose of mineral nitrogen has been applied at the commencement of an experiment, the initial concentration of mineral nitrogen in the medium is not maintained, but is progressively decreased by assimilation during the growth of the plant. Since nodules can be produced throughout the growth period, those nodules which appear towards the end of the experiment have been exposed to a lower concentration, actual or effective (see pp. 178, 179) of nitrate, than have the earlier nodules. The duration of an experiment, and the amount of growth made by the plants, must thus affect the results obtained. In comparing results of different experiments, it is necessary to remember, too, that the mineral nitrogen may have been applied in a single dose, or repeatedly.

It is not surprising to find that different authors have obtained variable results in attempts to ascertain the doses of mineral nitrogen

that would inhibit, or reduce, nodule formation. Most of this work has been carried out with the nitrates of sodium, potassium, calcium, and ammonium, and with ammonium sulphate. (The use of ammonium nitrate seems unnecessarily to complicate the issue.) These compounds have been found to stop nodule formation in solution and in agar culture at doses varying from 5 to 175 mg. of N per litre (Hiltner, 1900; Marchal, 1901; Flaman, 1903-4; Hills, 1918), the mean figure being about 60 mg. N per litre. A number of workers have attempted to fix the dose of mineral nitrogen that will stop nodule formation in sand and in soil. In cases where the published experimental details enable the initial concentration of mineral nitrogen to be estimated (Fred and Graul, 1916; J. K. Wilson, 1917; Hills, 1918; Strowd, 1920; Ohkawara, 1928), the inhibiting doses ranged from 21 to 488 mg. N per litre of moisture, with a mean of about 185 mg. There is thus some justification for the view generally held, that larger doses are needed to stop nodule formation in soil and in sand than in water culture, but the discordant results, as well as the differences in the mode of application of the nitrogen compounds, make the evidence rather questionable.

Several workers have observed that nodules tend to be of smaller size on plants supplied with mineral nitrogen, than on control plants (Giöbel, 1926). Far less attention has, however, been given to this effect of mineral nitrogen upon the size, than to its effect upon the presence or number of nodules.

A number of theories have been put forward to account for this inhibiting effect of nitrates upon nodules. Several authors have found that the concentrations of nitrate that inhibit nodule formation are not harmful to the organisms outside the plant. Müller and Stapp (1926), for example, found that 0.2 per cent. by weight of potassium, sodium, or ammonium nitrate was actually stimulating to nodule bacteria in culture. At the conclusion of the experiment described below we isolated bacteria by plating from a few nodules obtained at harvest on October 8, from plants in pots of series A, E and F, receiving 0, 6.0, and 10.0 gm. of sodium nitrate, respectively. On November 4 all plates showed colonies of the same type as the strain originally used; there were no visible differences due to the previous presence or absence of nitrate in the sand. This finding supports the more elaborate investigations of Müller and Stapp (1926), Prucha (1915), Hills (1918), and Giöbel (1926), to the effect that reduction in nodule numbers on plants grown in presence of nitrate is not due to any effect of the nitrate upon the nodule bacteria *per se*.

It seems clear, therefore, that nitrate must act through the agency of the host plant. Hiltner (1900) attributed the effect to a general enhanced vigour of the plant. This theory seems to be disproved by J. K. Wilson's experiment (1917), in which he separated the root system of a soy bean plant, placing one-half in nitrate solution, and showed that the other half, immersed in nitrate-free solution, developed nodules normally, thus showing that the effect of nitrate is local.

Stowd (1920) thought that the nitrates within the plant attained a concentration toxic to the nodule bacteria, but this theory has been criticised by Giöbel on the grounds that Stowd had miscalculated the concentration of nitrate that is toxic.

More recent opinion has tended to stress the change in carbon-nitrogen ratio, produced within the plant by nitrate absorption. The earliest theory based upon this change appears to be Mazé's (1898), according to which a plant deficient in nitrogen contains an excess of carbohydrates, a part of which is excreted by the root hairs, and attracts the nodule bacteria. This excess and excretion of carbohydrates will, in a plant adequately supplied with nitrates, be prevented by the building up of protein.

Hopkins and Fred (1933) and Fred and P. W. Wilson (1934) correlated the formation and activity of nodules with factors increasing the carbohydrate concentration in the plant; they connected the inhibiting effect of nitrates with a lowering of the carbon-nitrogen ratio within the plant, leading to a decrease in the concentration of carbohydrates. Fred and P. W. Wilson (1934), indeed showed that the effect of nitrate could be mitigated by increasing the  $\text{CO}_2$  content of the atmosphere surrounding the plants. They did not, however, explain the mechanism by which the altered carbon-nitrogen ratio affects either the infection of the roots or the formation and activity of the nodules.

Allison and Ludwig (1934) showed that sucrose added to sand cultures of lucerne tended to overcome the injurious effects of nitrate. They suggested that the normally harmful effects were due to reduced root growth, caused by insufficient carbohydrate supply to the roots.

Giöbel (1926) held a somewhat different view. According to him, the supplying of nitrate must decrease translocation of organic nitrogen compounds from the nodules. He believed that the accumulation of such compounds in the nodule became toxic to the bacteria.

The majority of these theories explain only some of the facts. Thus, Mazé's theory does not explain the reduced size of nodules under the influence of nitrates; Giöbel's does not explain the reduced number of



nodules formed, while Allison and Ludwig's implies that the number and size of nodules should be proportionate to the root growth.

Before the existing theories can be evaluated, or their numbers usefully increased, it seems necessary to determine firstly, whether nitrates act principally in checking infection of the root or by reducing the growth of the nodules; and secondly, whether there is any adequate correlation between the reduced nodulation and the root growth.

The pot experiments with lucerne here described were undertaken in 1932 with the object of determining quantitatively the relative effect of increasing doses of nitrate: (a) upon the numbers of nodules formed, using that as a measure of root infection; (b) upon the size of the nodules; (c) upon the root development and the correlation of that to nodule number and size.

#### PLAN OF EXPERIMENT

Glazed pots holding about 12 kg. of clean river sand were used in an unheated greenhouse. There were four parallel pots for each of six treatments, consisting of the addition of sodium nitrate in amounts of from 0 to 10 gm. to each pot. Inoculated lucerne (*Medicago sativa*, var. Grimm) was grown: seedlings, previously sown in three additional "nursery" pots, nitrate-free, were transplanted when a few days old, at the rate of ten seedlings per experimental pot. Two reapings of tops were made: the first a cutting made without disturbing the roots, and the second when the sand was emptied out to harvest both tops and roots. Yields and nitrogen determinations, recorded separately, for tops and for roots were made from each pot, and on a few samples of sand at the end of the experiment. Nodules were counted, measurements of length were made, and an estimate of "bacterial tissue" was calculated as described later.

#### GREENHOUSE TECHNIQUE

Each of the twenty-four experimental and three "nursery" pots received an admixture of 10 gm. of precipitated calcium carbonate.

Each pot received 1 litre of food solution of the following composition:

Distilled water to 1 litre		FePO <sub>4</sub> ...	...	5 gm.
KH <sub>2</sub> PO <sub>4</sub> ...	10 gm.	CaH <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub> ...	...	5 gm.
MgSO <sub>4</sub> .7H <sub>2</sub> O ...	10 gm.	MnSO <sub>4</sub> cryst. ...	c. 16 mg.	
NaCl ...	5 gm.	H <sub>3</sub> BO <sub>3</sub> ...	...	10 mg.

Ferric citrate 1.0 gm. suspended in citric acid 1.0 gm. in solution; FeCl<sub>3</sub>, 10 per cent. solution, 1.1 ml., and a trace of sulphuric acid.

The following dressings of sodium nitrate dissolved in the litre of food solution were given to each series respectively: series A, no nitrate; B, 1.0 gm.; C, 2.0 gm.; D, 4.0 gm.; E, 6.0 gm.; F, 10.0 gm. Since the sand ultimately contained about a second litre of liquid these doses represent a range of concentrations of from about 80 to 800 mg. of  $\text{NO}_3\text{-N}$  per litre in the solution available to the roots. No further application of artificial nitrogen was made after this initial dose given with the food solution on June 29 and 30, when ten seedlings, each having one true leaf open, and some lateral roots forming, were transplanted into each of the twenty-four pots.

On July 4 each pot was inoculated with 100 ml. of a thick suspension of an active strain of lucerne nodule bacteria. On July 8 and 9 a further litre of food solution, nitrogen-free, was added to each pot. Randomisation was adopted throughout in the positioning and treatment of pots.

### GROWTH

By August 12 there was marked increase of growth due to the nitrate supplied, although series F was not growing as well as series B-E. Controls were very weak, and all series were reinoculated. Thereafter growth proceeded satisfactorily. The differences in growth related to the nitrate dressings, persisted until September 14, as the yields of clippings show (Table I): but the differences then appeared to be less marked than on August 12. In the second growth of tops (reaped on October 8, at the end of the experiment) the series with no nitrate and that with 1 gm. of nitrate had the best top growth; these series also had the greatest root reserves. All plants in one pot of series E were then dead, and another pot of that series had only three living plants.

Results of yield of dry matter for the two reappings are given in Table I, and nitrogen determinations in Table II.

Table I. *Dry weights of lucerne tops and roots*  
(mean weights per pot in gm.)

Series	$\text{NaNO}_3$ gm.	First reaping Sept. 14, clipping of tops only	Second reaping Oct. 8		S.E. (roots)	Tops, both reappings S.E.
			Tops	Roots		
A	0	2.03	2.83	2.91	$\pm 0.47$	$4.86 \pm 0.39$
B	1	2.22	2.99	3.43	$\pm 0.46$	$5.21 \pm 0.18$
C	2	3.07	2.28	2.09	$\pm 0.55$	$5.35 \pm 0.45$
D	4	4.23	1.98	1.59	$\pm 0.45$	$6.21 \pm 0.56$
E	6	3.66	1.37	1.24	$\pm 0.45$	$5.03 \pm 0.52$
F	10	2.88	2.08	2.28	$\pm 0.39$	$4.96 \pm 0.44$

S.E. = standard error.

Table II. *Nitrogen in dry matter of lucerne tops and roots and in sand (mean results per pot)*

Series	N mg. given initially as NaNO <sub>3</sub> *	Tops, both reappings†		Roots		Total N mg.	S.E.	N in sand at end of exp.	
		N %	N mg.	N %	N mg.			%	mg.
A	0	4.29	208.6	2.67	77.8	286.4	±21.5	—	—
B	163	3.42	178.2	2.16	74.0	252.2	± 9.6	0.00067	87
C	326	3.25	173.9	2.62	54.8	228.7	±23.8	—	—
D	651	4.01	249.3	2.97	47.3	296.6	±34.1	—	—
E	977	4.51	227.0	3.22	39.9	266.9	±31.4	—	—
F	1628	4.81	237.7	2.93	66.7	305.4	±32.9	0.00439	543

\* A small allowance made for moisture (Thornton and Nicol, 1934 a).

† Bulkcd for nitrogen determinations.

#### THE EFFECT OF NITRATE UPON GROWTH, NODULE NUMBERS, AND NODULE LENGTHS

Repressive effects with increasing doses of nitrate showed themselves most clearly in reduced numbers of nodules and in reduced nodule lengths. With other indices of growth, such as dry matter, nitrogen percentage, and total nitrogen, the effects were either substantially independent (as total N, or ratio of tops to roots at harvest) of the amount of nitrate added, or else tended to have a maximum at about the level of 1.4 gm. of NaNO<sub>3</sub>. When there was a maximum (as with the yields of clippings) it was seldom pronounced.

The mean root weights showed with increasing doses of nitrate a decline that is just statistically significant<sup>1</sup>.

The values of the yields of tops divided by the yields of root were almost constant, with a mean value of 1.03, if the October harvest alone is considered, but if the yields of the September clippings are added in, the ratio increases with increasing nitrate. The tops/roots ratio in our 1931 experiment (Thornton and Nicol, 1934 a) with lucerne grown alone in presence of initial doses of 0.33, 1.0 and 3.0 gm. of NaNO<sub>3</sub> showed a smaller increase (over a smaller range of nitrate dressings) in the same sense at each of the four complete harvests of tops together with roots made in that year. The 1931 experiment was not complicated by clipping. Clipping, however, according to another experiment made by us in 1933 (Thornton and Nicol, 1934 b), has no effect upon the nodule numbers or lengths, at least in absence of nitrate.

<sup>1</sup> Degrees of freedom are: 5 for treatments and 18 for error (23 in all). The value of  $z$  calculated from the results for each pot is 0.543, which is slightly above the 5 per cent. point of probability (Fisher, 1932).

The total nitrogen in the plants remained practically constant at about 280 mg. per pot, while the doses of nitrate-nitrogen increased from 0 to 1628 mg. Hence, with doses above 1.0 gm.  $\text{NaNO}_3$ , there was a loss of nitrogen. This loss is evident on looking at Table II. After 10 gm.  $\text{NaNO}_3$  (1628 mg. N) had been applied at the commencement of the experiment, there was a recovery of 305 mg. in the plant and 543 mg. in the sand (including any traces of N originally present); the balance, 780 mg., could not be accounted for<sup>1</sup>. It is impossible also to make any estimate of the amounts or proportion of nitrogen that had been fixed by the plants.

Compared with the plants that had no artificial nitrogen, those that had 1.0 gm. of sodium nitrate manifested a small and non-significant rise in mean nodule numbers (Table III). Thereafter, with increasing doses of nitrate, the nodule numbers dropped sharply but remained unchanged as between 6.0 and 10.0 gm.  $\text{NaNO}_3$ .

No concentration of nitrate tried inhibited nodule production entirely. The highest dose represented an initial concentration of about 1600 mg. of  $\text{NO}_3\text{-N}$  per litre of solution in the sand. Though higher than the "inhibiting" doses of other authors (see p. 174) our figures are comparable with that of M. P. Löhnis (1930), who found that a concentration of 280 mg.  $\text{NO}_3\text{-N}$  per litre reduced nodulation in agar cultures to about one-half.

The mean nodule length was, however, reduced by 1 gm. sodium nitrate to a value only two-thirds of that of the nodules on the plants receiving no nitrogen, and the mean lengths almost reached a minimum at 4.0 gm.  $\text{NaNO}_3$ , thereafter decreasing slightly to a value about one-third of the controls. A steep and immediate drop in the nodule lengths set in after an initial dressing of only 163 mg. of nitrate-nitrogen in 12 kg. of sand. This dose is comparable with an ordinary agricultural dressing.

Effects of the various doses of nitrate upon overall lengths of nodules

<sup>1</sup> It has been pointed out by Zinzadzé (1932) that, in conditions of deficient aeration, nitrate solutions form nitrite, which is toxic to plants. This formation of nitrite has been noted by us during the germination of lucerne seeds in aqueous nitrate solutions in small test-tubes. The formation of nitrite might provide a partial explanation for the undesirable effects of nitrate upon legumes, particularly in water cultures. It seems unlikely that aeration in sand cultures is defective, though in a paper of Virtanen and v. Hausen (1935) evidence is presented to the effect that in water cultures aeration is a limiting factor in the production of nodules.

The formation of nitrite in sand cultures would not account directly for the disappearance of nitrogen from the sand, unless it be assumed that the missing nitrate was completely reduced to gaseous nitrogen.

are graphically set forth by the percentage frequency diagram, Fig. 1. The actual form of the "steps", and also the mean values, are shown for the control plants (without nitrate), but, for the sake of clearness, only the mean values are plotted for the other curves. The curves are roughly parallel; the extreme values progressively decrease with increasing amounts of nitrate.

Length is not a precise basis for the comparison of the activity of nodules, even at equal ages. It may be remarked that the real ages of

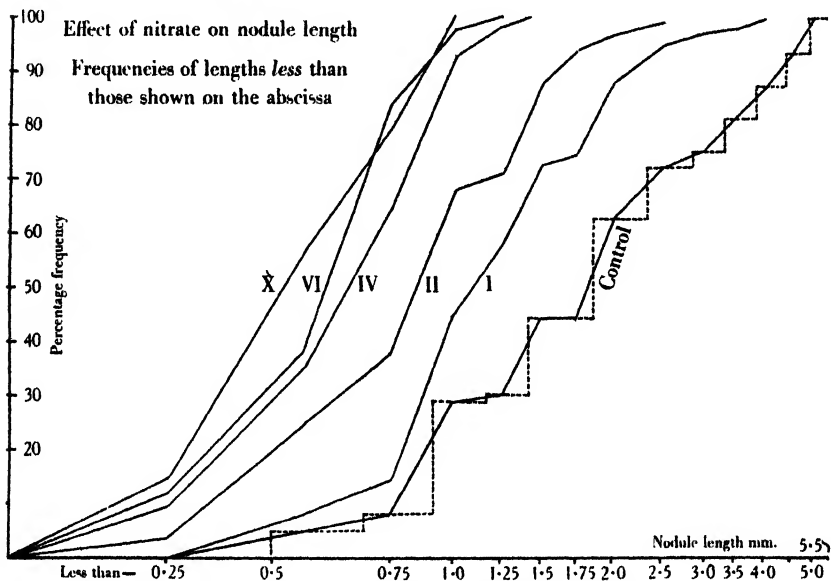


Fig. 1. Frequencies of nodule lengths, expressed as percentages of the mean total number of nodules per pot. The graphs indicate the percentage frequencies of nodules of length less than those given as abscissae.

the nodules in different series may not have been equal, owing to the considerations discussed on p. 173.

From the geometrical principle of similar figures, it might be expected that the activity of a nodule should be proportional to its volume, and therefore to the cube of its linear dimensions. Actually, the whole of a nodule is not filled with bacteria. Although the site of the nitrogen-fixing process in the nodule is not known with certainty, it may reasonably be assumed to be that region, in which the majority of nodule tissue cells are filled with bacteria. This region hereafter described as the "bacterial tissue", is homogeneous and is approximately cylindrical in form in most lucerne nodules, though the ratio of axial length

to diameter varies from nodule to nodule. The relation of bacterial tissue to nodule length is therefore not simple, and, with small nodules at least, we have found it to be far from linear and close to the cubic relation.

Table III. *Mean nodule numbers and mean nodule lengths per pot (3, 6, 8, 9, 10 or 11 plants—corrected to 10 plants per pot)*

Series	Number of nodules per pot	S.E.	Nodule lengths mm.	S.E. mm.	Number of nodules measured for length	Number of nodules per gm. of root
A	496	± 27	2.22	± 0.057	119	178
B	508	± 67	1.42	± 0.095	120	145
C	333	± 44	1.03	± 0.13	100	152
D	204	± 27	0.705	± 0.039	90	100
E	69	± 41	0.65	± 0.057	49	42
F	68	± 22	0.59	± 0.076	54	29

The degree of correlation between overall nodule length and the volume of bacterial tissue was investigated with the following technique: Nodules were stained whole with Grenacher's borax carmine, and differentiated with acid alcohol until the bacterial tissue showed by transparency. They were then brought by stages into absolute alcohol and finally cleared and mounted in cedarwood oil. The nodules prepared in this way were examined microscopically with a micrometer eyepiece. Length and diameter of the bacterial tissue were measured, and the volume calculated, on the assumption that the bacterial tissue was a cylindrical mass. Frequently, one or both ends of the mass of bacterial tissue appeared as a sharply defined straight line perpendicular to the axis, but where the boundary appeared hemispherical, a convention was adopted in fixing the position of the equivalent diametral (or, more rarely, lateral) boundary. The sides of the mass of bacterial tissue were usually straight, so that it was thought that the cylindrical form was a closer approximation to the true form than any other—such as the ellipsoidal.

After more than one hundred nodules from a number of sources, including both agar and sand cultures, had thus been measured, it was found that their overall length and the volume of bacterial tissue they contained were connected by relations shown graphically in Fig. 2.

The full and dotted curves on Fig. 2 require a few words of explanation. The full curve was obtained in the ordinary way by drawing the curve which best fitted the plotted points indicating the observed relations between nodule overall lengths and volumes of bacterial tissue,

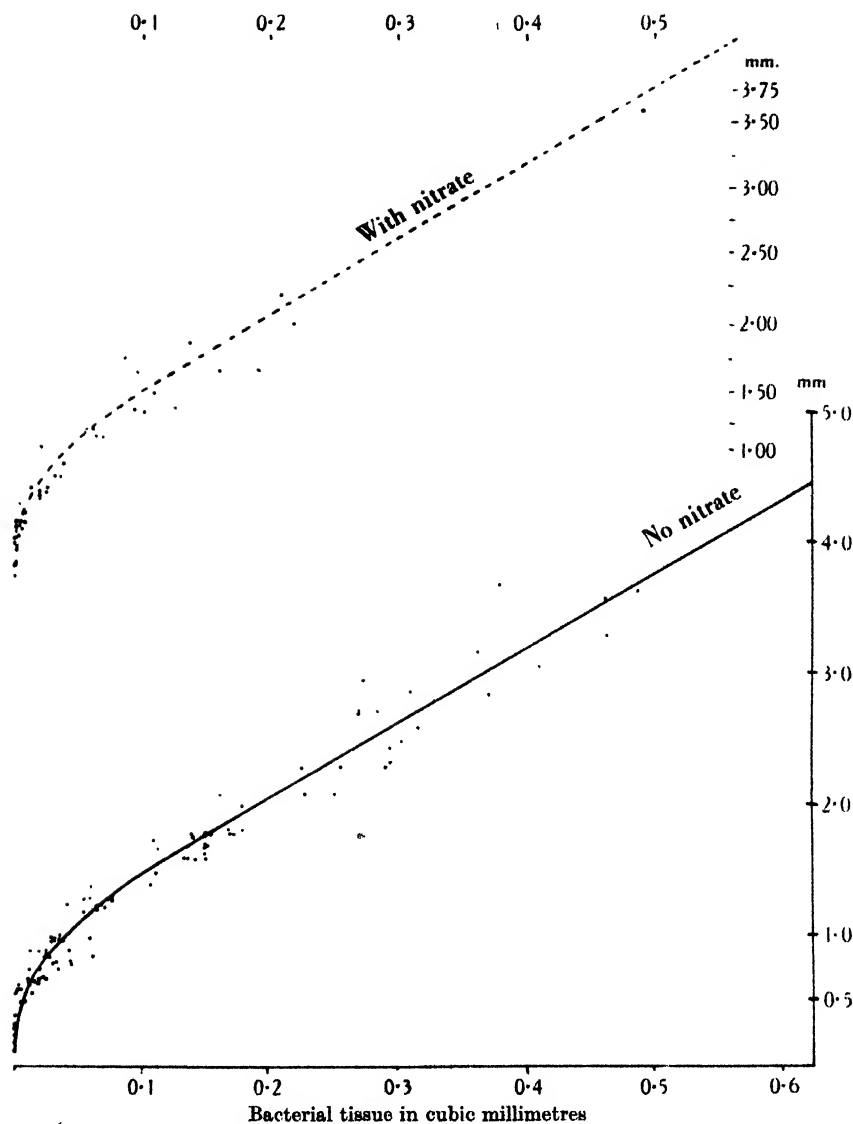


Fig. 2. Volumes of bacterial tissue in cubic mm., in nodules of various lengths grown in absence and in presence of sodium nitrate. Two points adjacent to the straight-line part of the lower curve occur beyond the portion shown.

in nodules on plants grown in absence of added nitrate. A number of points were also plotted in the upper part of Fig. 2 for the relation between nodule overall lengths and volumes of bacterial tissue in nodules on plants grown in presence of various doses of sodium nitrate. The full curve already obtained for the "no nitrate" nodules was moved exactly parallel to itself into the position shown by the dotted curve. It was then found to represent the best fit among the "nitrate"-nodule points.

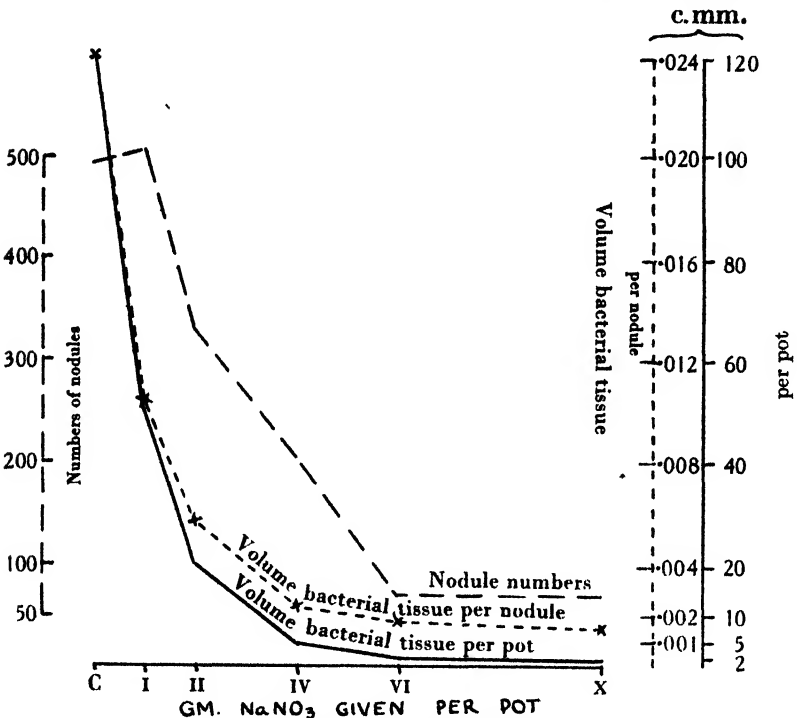


Fig. 3. Mean values for nodule numbers, volume of bacterial tissue per nodule, and volume of bacterial tissue per pot.

This furnishes an incisive proof that the relation between nodule lengths and volumes of bacterial tissue in lucerne nodules is independent of the presence of nitrate.

It is thus permissible to use this curve to convert measurements of overall nodule lengths into volumes of bacterial tissue even where the nodules measured are from plants variously dosed with nitrate.

The estimates of bacterial tissue volumes for the pot experiments are shown in Fig. 3. The volume of bacterial tissue per nodule was more adversely affected by applications of nitrate than were either the nodule



numbers or the nodule lengths. Thus, while the addition of 1 gm. of sodium nitrate reduced the mean nodule length by about one-third as compared with the controls, it reduced the mean bacterial tissue *per nodule* to less than one half. With 4 gm. of sodium nitrate the mean nodule length was reduced by about two-thirds, but the volume of bacterial tissue *per nodule* was reduced to about one-tenth of that in the controls.

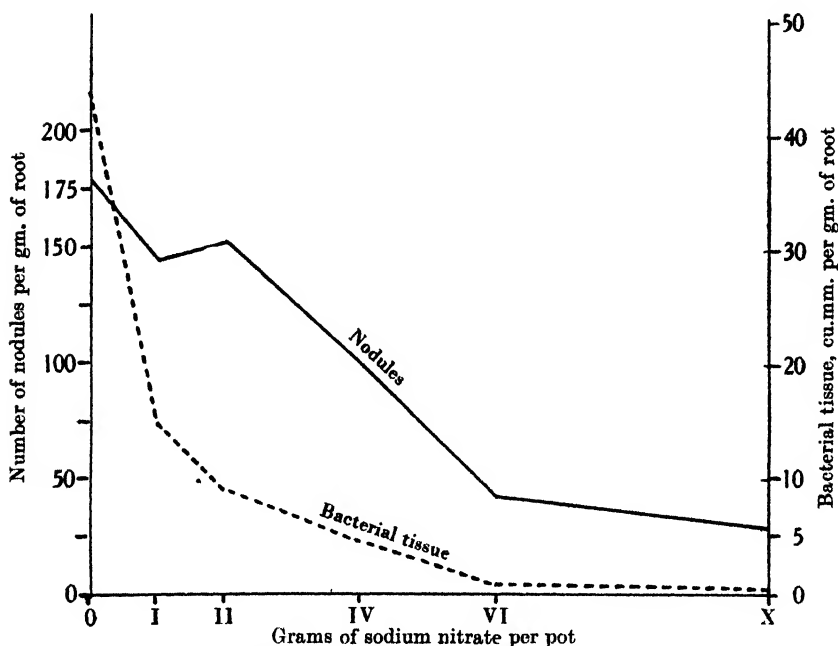


Fig. 4. Bacterial tissue, and nodules, per gram of root.

The mean bacterial tissue *per pot* was consequently greatly reduced even by 1 gm. of nitrate, but, with larger doses of nitrate, the mean volume of bacterial tissue *per pot* was decreased to a still more striking extent, owing to the combined effects of lessened numbers and smaller size of nodules.

As already mentioned on p. 178, there was a slight reduction in the root dry matter with increasing doses of nitrate, but in presence of the higher doses of nitrate the reduction in the number and size of nodules *per gram of root* was very much more pronounced than the reduction in root tissue alone.

Fig. 4 shows the nodule numbers, and volume of bacterial tissue, *per gram of root* for the 1932 experiment.

It will be seen that in the range 0–2 gm. of  $\text{NaNO}_3$ , the fall in the number of nodules per gram of root was small, and that it became appreciable at and above the level of 4 gm. of  $\text{NaNO}_3$ .

The volume of bacterial tissue per gram of root was (in 1932) reduced to one-third by only 1 gm. of  $\text{NaNO}_3$ , and was reduced to about one-sixtieth of the controls by the 6-gm. dose.

In these experiments, then, sodium nitrate depressed both the number of nodules and their size. Our experiments thus confirmed the results

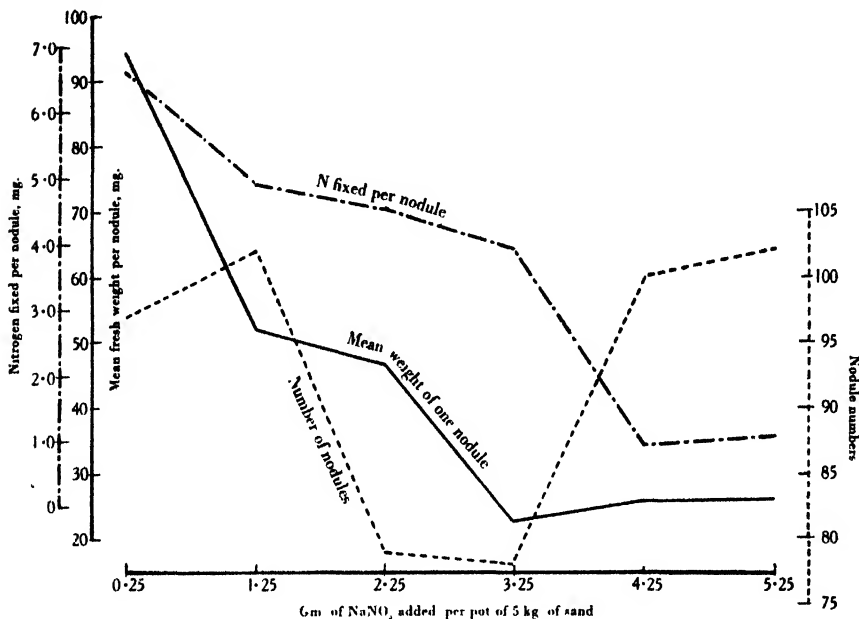


Fig. 5. Effects of sodium nitrate upon inoculated soy beans: data of Giöbel (1926). Giöbel's nitrate doses, originally expressed as pounds per acre, have been recalculated.

obtained by Giöbel (1926), who made a series of experiments to test the effect of sodium nitrate upon inoculated soy bean plants, and recorded the numbers and fresh weights of nodules on plants supplied with different doses of nitrate. Some results of Giöbel's (second) experiment with soy beans are graphed in Fig. 5. The experiment showed a rapid diminution in the mean size of nodules as the nitrogenous dose increased. The effect upon nodule numbers was less regular, there being a considerable increase in numbers of small nodules with the two highest doses.

In the same experiment Giöbel also estimated the amounts of nitrogen fixed by plants supplied with nitrate, as compared with uninoculated

control plants; the amounts fixed per nodule were roughly proportional to the nodule size. His other experiment with soy beans gave less regular results, but in general they supported the view that the effect of nitrate in reducing nodule size is at least as important as is its influence on nodule numbers. Fred and P. W. Wilson (1934) studied the effect of sodium nitrate on the numbers and weights of nodules on clover, and confirmed this conclusion.

#### DISCUSSION

The data from our own and similar experiments enable the existing theories of nitrate effect to be reviewed more critically.

The fact that the growth of the individual nodule is reduced by nitrate, renders inadequate any theory of nitrate effect such as Mazé's, which involves a supposed action outside the plant. On the other hand, such theories as Giöbel's, which attribute the effect to a change inside the nodule, seem inadequate to account for a checking of infection leading to reduced nodule numbers. The hypothesis of Allison and Ludwig (1934), attributing nitrate effect to reduced root growth, implies a correlation between nodule and root development, but in our experiments in 1931 (Thornton and Nicol, 1934 *a*) and 1932 (described here), there was no clear connection between these characters. Indeed, in the present experiment both the nodule numbers, and the volumes of bacterial tissue calculated per unit mass of root, rapidly fell off with increasing nitrate (Fig. 4).

In this experiment sodium nitrate manifested a greater effect upon the volume of bacterial tissue than upon nodule numbers. This was particularly the case with small doses of nitrate: 1 gm. of sodium nitrate (163 mg. N) was without effect upon nodule numbers, but halved the bacterial tissue. It appears, then, that a smaller dose of nitrate is needed to interfere with the development of nodules already formed than is required to check infection of the roots. There would thus appear to be two problems, involved with the nitrate effect, which need separate examination: (*a*) the influence of nitrate upon root-hair infection; (*b*) the influence of nitrate upon the growing nodule. These two problems will form the subjects of subsequent papers.

#### SUMMARY

A sand-culture experiment with inoculated lucerne was made to test the effect of increasing doses of sodium nitrate upon the yield and nitrogen content of tops and roots, and upon the number and develop-

ment of nodules. The pots were divided into six series receiving from 0 to 10 gm. of sodium nitrate per pot containing 12 kg. of sand.

There was no correlation between yield or nitrogen content of the lucerne, and the dose of nitrate.

The number of nodules, though unaffected by 1 gm. of sodium nitrate, was progressively decreased by the stronger doses.

The length of nodules was reduced about 30 per cent. by 1 gm. of sodium nitrate, and decreased progressively with stronger doses.

Nodules were found to show a definite relationship between their overall length and the volume of contained bacterial tissue. Using this relation, the mean volumes of bacterial tissue per nodule and per pot for each series were calculated. The reduction effected by nitrate was far greater when measured in this way, owing to the small contribution of bacterial tissue derived from nodules less than 1.5 mm. in length.

Increasing doses of nitrate also progressively decreased the nodule numbers, and the content of bacterial tissue, per gram of root. The effect of nitrate was thus not due to reduced root growth.

#### ACKNOWLEDGMENT

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# THE NUTRITIVE VALUE OF ARTIFICIALLY DRIED GRASS AND ITS EFFECT ON THE QUALITY OF MILK PRODUCED BY COWS OF THE MAIN DAIRY BREEDS

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## INTRODUCTION

THE artificial drying of grassland herbage and of other forage crops has recently assumed considerable importance.

That artificial drying can produce a material of high nutritive value, as judged from chemical analysis and digestibility trials, has been shown by Woodman (1), Watson (2, 3), Hodgson and Knott (4), and Newlander and Jones (5), among others.

It has also been shown (6) that artificial drying does not materially affect the digestibility of the various constituents. Another important constituent of the green crop—the carotene—is retained in almost unimpaired amount by artificial drying (7). Recent work (8) has demonstrated that the exposure of *dried* grass even to relatively low temperatures may result in a depression of the digestibility of the protein, and of the carotene content, the magnitude of the effect depending on the length of exposure and on the temperature.

In an efficient drier, however, the material is removed as soon as it is dry, and no appreciable depression of digestibility or loss of carotenoid pigments occurs.

The data available on feeding trials are not extensive. A preliminary experiment carried out at Jealott's Hill in 1930 (2) with dairy cows showed that artificially dried grass was of high feeding value and could replace the greater proportion of the production ration without affecting the milk yield. An experiment carried out by Newlander (9) showed that the complete replacement of the concentrates by artificially dried grass caused a fall in the yields of milk and butterfat, whereas a partial replacement was satisfactory. Where the hay was partially replaced by an equivalent quantity of nutrients as artificially dried grass, the yields improved.

An important effect of the inclusion of artificially dried grass in the ration of the dairy cow during the winter stall-feeding period is the effect on the colour of the milk. It has been shown (7, 10) at Jealott's Hill that the inclusion of artificially dried grass in sufficient amount in the ration at this time will increase the yellow colour of the butterfat to the level usually associated with the milk of cows when at pasture.

It has further been demonstrated that the yellow colour is positively correlated with the carotene content of the butter, and with its vitamin-A potency.

There were indications, however (10), that there exists a "ceiling" value for the breeds thus far examined—Ayrshire and Shorthorn—beyond which it is impossible to improve the yellow colour of the milk.

The position to date may then be summarised somewhat as follows:

Owing to the difficulties inherent in feeding trials with dairy cows, no data exist which are capable of statistical interpretation in regard to the relative merits of artificially dried grass and other foodstuffs in use on the farm.

Another point at issue is the relative efficiency of the main dairy breeds in passing the food carotene into the milk. It has been shown, for example (10), that on rations containing equal amounts of carotene, the Shorthorn cow produces a milk of higher carotene content than does the Ayrshire.

It was decided, therefore, to carry out an experiment over the winter of 1933-4 which might throw some light on these points.

#### DETAILS OF THE EXPERIMENT

The experiment was of the change-over type and there were two main groups of ten cows each. Each group consisted of five subgroups of two cows. It was decided in the first place to use cows of five different dairy breeds. It was, however, not practicable to obtain animals of one of the breeds selected so two subgroups of Shorthorn cows were allocated to each main group.

There were four breeds represented, Guernsey, Ayrshire, Friesian and Shorthorn. Four animals were available from each of the first three breeds and eight Shorthorns.

The cows were then allocated at random to one or other of the two groups, A and B.

The names and details of the cows are given in Table I, together with their allocations. Those marked as having entered the herd at a

certain date were bought as freshly calved cows, and in most cases it was not possible to get actual calving dates, though they would probably have been in milk not more than 1-2 weeks at the date of purchase.

Table I. *List of cows*

Group A			Group B		
Breed	Cow	Calved	Breed	Cow	Calved
Shorthorn	Betty	21. ix. 33	Shorthorn	Queenie	Entered 22. ix. 33
	Hollyhock	26. xi. 33		Kathy 3	21. ix. 33
Friesian	Pearl	Entered 25. xi. 33	Friesian	Dairymaid	Entered 25. xi. 33
	Saucy	Entered 25. xi. 33		Fancy	Entered 25. xi. 33
Ayrshire	Ninna	10. xi. 34	Ayrshire	Grace	20. x. 34
	Tiny	16. ix. 34		Ruth	16. x. 34
Guernsey	Nellie	Entered 16. xi. 33	Guernsey	Eva	Entered 16. xi. 33
	Bessie	Entered 16. xi. 33		Lottie	Entered 16. xi. 33
Shorthorn	Olive	Entered 22. ix. 34	Shorthorn	Cherry	Entered 22. ix. 33
	Martha	20. xi. 33		Gladys	Entered 3. xii. 33

It was evident from the dates of calving that all the cows might be expected to remain in milk during the 20 weeks over which the experiment would extend.

After the sixth week in milk the yield falls more or less gradually, particularly during the winter when no pasturage is available. The yields might, therefore, be expected to fall at a fairly regular rate throughout the period of the experiment.

After a preliminary period during which all the cows were fed alike, the experiment was started on December 10, 1933. The durations of the periods were:

- Period 1. December 10, 1933 to January 14, 1934.
- „ 2. January 14, 1934 to February 18, 1934.
- „ 3. February 18, 1934 to March 25, 1934.
- „ 4. March 25, 1934 to April 29, 1934.

The cows were all housed together in one-half of a cowshed. They stood back to back on each side of a central passage, ten on each side. On one side were the four Guernseys and six Shorthorns; on the other side were the Friesians, the Ayrshires and two Shorthorns.

The four Guernseys stood in adjacent stalls, as did the Friesians and also the Ayrshires. Within each breed the two cows of each subgroup stood together for ease of feeding. There were three pairs of Shorthorn cows on one side, and one pair on the other side of the gangway.



## RATIONS FED

The foodstuffs used during the course of the trial were sampled weekly, and where the material could be stored a composite weighted mean sample was made up at the end of the period for analysis. The analytical figures are summarised in Tables II and III.

Table II. *Composition of foodstuffs used in the experiment*

	Kale %	Cab- bage %	Man- golds %	Rice meal %	Concen- trates %	Hay (periods 1-4) %				Dried grass (periods 1-4) %			
						1st	2nd	3rd	4th	1st	2nd	3rd	4th
Moisture	82.6	90.9	88.9	9.23	9.26	18.2	14.1	14.8	12.9	7.73	10.69	9.89	10.53
Ether extract	0.52	0.19	0.13	14.96	7.83	1.48	1.54	1.57	1.63	3.75	3.02	3.02	3.23
Fibre	1.79	1.05	0.72	5.75	5.84	24.60	24.39	24.78	28.28	21.08	20.46	20.43	20.61
Crude protein	3.64	1.44	1.14	11.38	21.25	7.99	7.66	8.13	6.71	16.00	15.31	16.25	15.50
Ash	1.97	0.90	1.34	8.34	6.72	5.99	6.26	6.73	6.24	7.47	7.13	6.94	7.35
N-free extract	9.48	5.52	7.77	50.34	49.10	41.74	46.04	43.98	44.24	43.97	43.39	43.47	42.78
CaO	0.36	0.04	0.04	0.07	0.63	0.70	0.72	0.68	0.47	1.08	1.07	1.01	1.03
P <sub>2</sub> O <sub>5</sub>	0.12	0.10	0.06	2.57	2.25	0.58	0.71	0.72	0.65	0.78	0.74	0.75	0.74
True protein	2.15	0.94	0.47	10.56	19.03	6.74	6.26	6.15	5.86	13.81	13.19	12.88	12.75
Ratio:													
True protein/ Crude protein	0.59	0.65	0.41	0.93	0.92	0.84	0.82	0.76	0.87	0.86	0.86	0.79	0.82

Table III. *Dried grass. Composition, digestibility and digestible nutrients*

	Average* composition %	Digestibility %	Digestible* nutrients %
Ether extract	3.60	43.43	1.56
Fibre	22.87	64.62	14.78
Crude protein	17.46	70.15	12.25
Ash	8.00	—	—
N-free extractives	48.07	69.52	33.42
Organic matter	92.00	68.06	62.62
True protein	14.57	65.61	9.56
Calcium (CaO)	1.16	—	—
Phosphoric acid (P <sub>2</sub> O <sub>5</sub> )	0.84	—	—
Ratio: True protein/Crude protein	0.83	—	—
Starch equivalent	—	—	52.88
Protein equivalent	—	—	10.91
Dry matter in fresh material	90.29	64.89	—

\* Stated as percentages of the dry matter.

The analytical values for the foodstuffs compare fairly well with average figures in the literature. The marrow-stem kale had a somewhat higher crude protein content than is usually associated with this crop, whilst the figure for cabbage is lower than published figures in regard to this constituent.

The mangolds, rice meal and concentrated mixture agree with average values. The concentrated mixture used was a proprietary cube, the exact proportions of the constituents being kindly supplied by the makers.

The hay, though it would be classed as good average meadow hay, showed a lower crude protein content than might have been expected.

The artificially dried grass was obtained from a multiple-band, low-temperature drier operating in the south-west of Scotland since, owing to the drought, no grass dried at Jealott's Hill was available. It was of lower crude protein content than was hoped for, but the digestibility was fairly high, though here, too, higher values had been expected. This was no doubt due to the dry conditions existing in 1933, when the grass was dried. As will be seen in Table XV, the colour of the dried grass was not as good as is usual for such material. It was, however, impossible to obtain a sufficient quantity of better quality dried grass that year.

The nutritive value of the various foodstuffs is summarised below. The digestibility of the artificially dried grass was determined experimentally, whilst for the other foodstuffs average figures were taken from the literature.

Table IV. *Nutritive value of foodstuffs*  
(percentage of the materials as fed)

	Starch equivalent	Protein equivalent	Digestible true protein
Kale	11.20	1.93	1.34
Cabbage	7.06	0.97	0.79
Mangolds	7.75	0.46	0.11
Concentrates	66.10	18.10	17.70
Rice meal	73.49	6.11	5.73
Hay, first period	28.46	2.95	2.52
„ second period	31.67	2.78	2.34
„ third period	30.03	2.87	2.30
„ fourth period	30.45	2.51	2.19
Artificially dried grass, first period	43.42*	10.06	9.02
„ second period	42.04*	9.62	8.62
„ third period	41.90*	9.84	8.42
„ fourth period	41.50*	9.55	8.33

\* Factor 0.58 used for fibre correction.

The actual amounts fed per cow per day are given in full in Table V as averages of the ration fed during each period.

It will be seen that 8 lb. of artificially dried grass were fed per head daily to all except the Guernsey cows, which received 10 lb. The dried grass was in meal form, and as such was not eaten readily by the cows. It had been hoped to replace the whole of the concentrated ration by artificially dried grass, but this proved impossible as the finely ground material was too dusty. The trouble was due to the state of division, since it has been found possible to feed larger amounts of dried grass in an unground condition.

Table V. *Average ration per day per cow per period in lb.*

Breed	Cow	First period				Second period				Third period				Fourth period				
		Hay	Kale or cabbage	Con-centrates	Dried grass	Hay	Man-golds	Con-centrates	Dried grass	Hay	Man-golds	Con-centrates	Dried grass	Hay	Man-golds	Con-centrates	Rice meal	Dried grass
Group A																		
Shorthorn	Betty	16	25	4½	8	17	30	9	—	17	30	2½	8	17	30	5	2½	—
	Hollyhock	16	25	7½	8	17	30	13½	—	17	30	8½	8	17	30	9	4½	—
Friesian	Pearl	18½	25	6½	8	19	30	11½	—	19	30	4½	8	19	30	5½	2½	—
	Saucy	17½	25	4	8	19	30	9½	—	19	30	2½	8	19	30	5	2½	—
Ayrshire	Ninna	16½	25	12	8	17	30	17½	—	17	30	10½	8	17	30	9½	4½	—
	Tiny	16	25	4	8	17	30	8½	—	17	30	2½	8	17	30	5	2½	—
Guernsey	Nellie	14	25	3½	10	15	30	8½	—	15	30	2½	10	15	30	5½	2½	—
	Bessie	16	25	6½	10	15½	30	9	—	15	30	1½	10	15	30	4½	2½	—
Shorthorn	Olive	14½	25	4	8	15½	30	8½	—	15	30	1½	8	15	30	5	2½	—
	Martha	16½	25	3½	8	17	30	9½	—	17	30	4½	4½*	17	30	5½	2½	—
Group B																		
Shorthorn	Queenie	14	25	8½	—	15	30	2½	8	15	30	7½	—	15	30	1½	—	8
	Kathy 3	17	25	13	—	18	30	5½	8	18	30	10	—	18	30	3	—	8
Friesian	Dairymaid	17	25	14½	—	18	30	7	8	18	30	11½	—	18	30	4½	—	8
	Fancy	16	25	13	—	17	30	6	8	17	30	11½	—	17	30	4½	—	8
Ayrshire	Grace	18	25	13	—	19	30	4½	8	19	30	8½	—	19	30	2	—	8
	Ruth	17	25	8½	—	18	30	2½	8	18	30	6½	—	18	30	1	—	8
Guernsey	Eva	14	25	12½	—	15	30	4	10	15	30	9½	—	15	30	2	—	10
	Lottie	14	25	11½	—	15	30	4½	10	15	30	9½	—	15	30	2	—	10
Shorthorn	Cherry	14½	25	10½	—	16	30	2½	8	16	30	7	—	16	30	1½	—	8
	Gladya	16	25	9½	—	17	30	4½	8	17	30	9½	—	17	30	3	—	8

\* Lost appetite for dried grass after the first week. The 8 lb. allowance was cut down to 4 lb. in the second week.

For the maintenance of the body weight of the cow, and to supply adequate energy for the usual body processes, an allowance, per 1000 lb. live weight, of 6.0 lb. of starch equivalent, containing 0.6 lb. of protein equivalent, is the standard in common use. This was adhered to as closely as was practicable, with the results shown in Table VI.

Table VI. *Starch equivalent and protein equivalent of ration fed to each cow per 1000 lb. live weight (stated in lb.)*

Name	First period		Second period		Third period		Fourth period		Average	
	S.E.	P.E.	S.E.	P.E.	S.E.	P.E.	S.E.	P.E.	S.E.	P.E.
Group A										
Betty	6.25	0.76	7.34	0.58	7.00	0.59	6.96	0.52	6.89	0.61
Hollyhock	6.34	0.77	7.48	0.59	7.06	0.59	7.05	0.53	6.98	0.62
Pearl	5.80	0.70	6.56	0.52	6.24	0.53	6.33	0.48	6.23	0.56
Saucy	5.92	0.69	6.50	0.52	6.13	0.52	6.26	0.47	6.20	0.55
Ninna	6.15	0.75	6.86	0.54	6.69	0.56	6.73	0.51	6.61	0.59
Tiny	6.42	0.78	7.48	0.59	7.32	0.62	7.24	0.55	7.12	0.64
Nellie	6.49	0.80	7.62	0.60	7.30	0.61	7.10	0.53	7.13	0.64
Bessie	6.27	0.77	7.74	0.53	6.29	0.52	6.31	0.47	6.40	0.57
Olive	6.17	0.76	7.01	0.55	6.47	0.54	6.49	0.48	6.54	0.58
Martha	—	—*	7.41	0.59	6.88	0.58	6.91	0.52	7.07	0.56
Average	6.20	0.75	7.10	0.56	6.74	0.58	6.74	0.51	6.71	0.60
Group B										
Queenie	6.70	0.83	8.00	0.63	7.21	0.60	7.20	0.54	7.28	0.65
Kathy 3	5.91	0.70	6.84	0.54	6.36	0.54	6.37	0.48	6.37	0.57
Dairymaid	5.79	0.69	6.63	0.53	6.28	0.53	6.36	0.48	6.27	0.56
Fancy	6.68	0.81	7.44	0.59	7.00	0.59	7.05	0.53	7.04	0.63
Grace	—	—*	7.77	0.62	7.70	0.66	7.69	0.58	7.72	0.62
Ruth	5.95	0.72	6.77	0.54	6.37	0.54	6.42	0.49	6.38	0.57
Eva	6.96	0.86	7.94	0.62	7.41	0.62	7.50	0.56	7.45	0.67
Lottie	6.56	0.81	7.53	0.59	7.07	0.59	7.19	0.54	7.09	0.63
Cherry	6.78	0.84	7.56	0.60	7.41	0.62	7.50	0.56	7.39	0.66
Gladys	6.75	0.82	7.91	0.63	7.34	0.62	7.55	0.58	7.39	0.66
Average	6.45	0.79	7.44	0.59	7.02	0.59	7.08	0.53	7.00	0.62

\* These cows were not weighed at the beginning of the first period.

In general, the starch equivalent allowance has been more liberal than was designed, but the protein equivalent is fairly close to the standard. This is the best that could be done with foodstuffs of this nature—the roughages.

In addition to the maintenance ration, the dairy cow is fed a ration of concentrated foodstuffs—the production ration—which is proportional to the milk yield of the animals. The standard suggested by the Departmental Committee on the Rationing of Dairy Cows (11) for a cow producing milk with an average butterfat content of 3.0–3.5 per cent. is 2.5 lb. of starch equivalent containing 0.6 lb. of protein equivalent per gallon of milk, whilst for a cow producing over 4.0 per cent. of butterfat in the milk the figures are 3.0 and 0.7 lb. respectively.

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There is evidence that these standards are high (12), and that the nutrients fed per gallon, and more particularly the protein equivalent, can be reduced considerably. It was therefore decided to cut down the allowance of starch equivalent to about 2.5 lb. for the Guernsey cows and to 2.0 lb. per gallon for the cows of the other three breeds, in order to prevent any intrinsically higher feeding value, that might be possessed by the dried grass, from being masked by the excess of nutrients provided by the standard allowances of starch and protein equivalents.

The figures in Table VII show that these levels were satisfactorily maintained.

Table VII. *Starch equivalent and protein equivalent fed per gallon of milk (stated in lb.)*

	First period		Second period		Third period		Fourth period	
	S.E.	P.E.	S.E.	P.E.	S.E.	P.E.	S.E.	P.E.
	Experimental		Control		Experimental		Control	
Group A								
Ordinary cows	2.06	0.49	2.07	0.57	2.12	0.50	2.16	0.44
Guernsey cows	2.57	0.60	2.23	0.61	2.54	0.56	2.41	0.50
Group B								
	Control		Experimental		Control		Experimental	
Ordinary cows	2.19	0.60	2.11	0.50	2.11	0.58	2.10	0.48
Guernsey cows	2.55	0.70	2.65	0.61	2.42	0.66	2.51	0.49

The protein equivalent content of the ration was also reduced in the case of the dried grass ration during the first period to a level of 0.6 lb. per gallon of milk for the Guernsey cows and 0.5 lb. for the other breeds, the control ration remaining at the usual level. In the second and third periods it was decided to cut both groups down to a level of intake of protein equivalent of 0.6 lb. per gallon for the Guernseys and 0.5 lb. for other breeds. The rations for the Guernseys conformed to this, but the protein equivalent in the dried grass ration was too high during these two periods. In the fourth period it was decided to cut down the protein equivalent still further to 0.5 lb. for the Guernseys and 0.45 for other breeds. The agreement between the group rations was more satisfactory in this last period.

It should be stressed that the aim of the experiment was not to test the effect of different levels of protein intake on milk yield, but to reduce the intake of nutrients to as low a level as possible consistent with the maintenance of a regular milk flow and prevent undue excess of nutrients.

## MILK YIELD

The cows were milked twice daily and the milk was weighed separately, for each cow, at every milking.

The individual weekly total yields are given in the Appendix, and the total weekly yields of the two groups are given in Table VIII.

Table VIII. *Total group yields (in lb. by weeks)*

Date	Group A		Group B	
	Dried grass	Control	Dried grass	Control
17. xii. 33	2656	—	—	2461
24. xii. 33	2566	—	—	2422
31. xii. 33	2534	—	—	2386
7. i. 34	2500	—	—	2322
14. i. 34	2403	—	—	2248
21. i. 34	—	2371	2171	—
28. i. 34	—	2315	2174	—
4. ii. 34	—	2312	2145	—
11. ii. 34	—	2331	2129	—
18. ii. 34	—	2297	2055	—
25. ii. 34	2242	—	—	2026
4. iii. 34	2186	—	—	1998
11. iii. 34	2156	—	—	1984
18. iii. 34	2115	—	—	1905
25. iii. 34	2064	—	—	1945
1. iv. 34	—	2014	1887	—
8. iv. 34	—	1952	1769	—
15. iv. 34	—	1892	1749	—
22. iv. 34	—	1883	1804	—
29. iv. 34	—	1863	1784	—

The figures for milk yield have been submitted to Mr M. S. Bartlett for statistical examination, and he has reported on them as follows:

“It may be observed that the existence of two groups is an essential feature of the experimental design if a valid estimate of error is to be made. If the results for one group only were available, it might be assumed as an approximation that the milk yields per cow were decreasing uniformly throughout the experimental period, and hence the average milk yield for one treatment might be compared with the mean of the average milk yields for the other treatment for the two periods before and after. The coincidence of one period with some weather factor, such as a cold spell, or any other systematic departure from the uniform decrease in milk yield would, however, invalidate any such comparison. The existence of the two groups renders such assumptions unnecessary, since any external cause influencing a particular period will be correspondingly reflected in the milk yields for the same period of the cows receiving the alternative treatment.

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"Thus if  $A_1$  refers to the average milk yield of a particular cow in group A for the first period, etc., then

$$y = (A_1 + A_3 + B_2 + B_4 - A_2 - A_4 - B_1 - B_3)/4$$

is a measure of the effect of one treatment over the other, a cow in group A with one in group B together making one unit. As has been mentioned above, each group consisted of five sub-groups of two—corresponding, except for a duplicate group of Shorthorns, to different breeds—and the two pairs corresponding to each breed were naturally associated together. Each pair was subdivided for the purpose of this analysis at random. In so far as the animals were not so positioned in pairs in the shed, the analysis is not strictly valid, but it is sufficient to add that though any observed differences might conceivably be due to position and not to treatment, since it will be seen that no differences were actually found, such possible causes of differences have not at any rate disturbed this particular experiment.

Table IX. *Summary of relative effect of treatments*  
(stated in lb. of milk per cow per week)

Breed	Cow A	Cow B	$y$ (Dried grass v. Control)
Shorthorn	Betty	Queenie	+ 4.90
	Hollyhock	Kathy 3	- 7.73
Friesian	Pearl	Fancy	+ 2.25
	Saucy	Dairymaid	- 4.70
Ayrshire	Ninna	Grace	+ 14.85
	Tiny	Ruth	- 6.08
Guernsey	Nellie	Lottie	- 1.65
	Bessie	Eva	+ 8.50
Shorthorn	Olive	Gladys	- 4.80
	Martha	Cherry	+ 2.55
		Mean	+ 0.81

"From the ten values of  $y$ , a standard error is calculated for the mean, and the following result obtained for the effect of Dried grass v. Control in lb. per week per cow.

Table X. *Summary of statistical analysis of milk yields*

	Dried grass v. Control	S.E.	Sig. diff. from 0
Lb. per week per cow	+ 0.81	2.28	5.16
% general mean	+ 0.38	1.05	2.40

"The mean effect 0.81 lb. per week is obviously not significant in view of the standard error calculated, and it may be concluded that the difference in treatments has had no observable effect on the quantity of milk produced. This can be regarded, moreover, as a real indication

of the equivalence of the treatments, since owing to the accuracy of the comparison, we should expect to have detected a difference of 2.4 per cent. in yield.

"The criticism might be made that any differences due to treatments had not time to show themselves in the 5-week periods of the experiment; the conclusion of null effect must therefore be qualified by due regard of the conditions under which this result was obtained.

"It is assumed in this analysis that if one treatment were in fact inferior, there would be little lag in the recovery of the milk yield in the succeeding period. If this assumption were not true, it would be possible for a difference to exist in spite of the results of Table X; this could appear in the differing slopes of the lactation curve for the different periods, rather than in the mean yields. A similar analysis (Table XI), however, on the drop in milk yield per period confirmed the conclusion of no significant treatment effect."

Table XI. *Summary of statistical analysis of weekly fall in milk yield*

Drop in yield	Dried grass v. Control	S.E.	Sig. diff. from 0
Lb. per week per cow	-0.37	0.61	1.38
% average drop	-9.5	15.7	35.6

It is evident from the statistical analysis of the milk yields that the artificially dried grass has replaced an equal weight of nutrients in the form of concentrated foodstuffs without affecting the performance of the cows. It may be assumed from this that the nutritive value calculated from the analytical values and the digestibility of the constituents gives a true measure of the value of artificially dried grass as a production ration for the dairy cow.

As has been suggested, it may be argued that the periods were too short to avoid a possible carry-over effect, but the uniformity of the yields suggests that this is not so, since it is well known that the dairy cow will react rapidly when changed to a lower plane of nutrition if she is in full yield.

It is, however, essential to settle this point and Bartlett, on the basis of these results (13), has suggested a technique of experiment allowing of long-period feeding trials which is being tested at Jealott's Hill at the present time.

#### COMPOSITION OF THE MILK

The fat and solids-not-fat contents of the milk were determined in the morning and evening milk on one day in each week. The fat was determined by the Gerber method; the solids-not-fat were calculated in



Table XII. *Tables showing fat and solids-not-fat per cow per 5-week period*

Group A																					
	Betty		Hollyhock		Pearl		Saucy		Ninna		Tiny		Nellie		Bessie		Olive		Martha		
	Fat	S.N.F.	Fat	S.N.F.	Fat	S.N.F.	Fat	S.N.F.	Fat	S.N.F.	Fat	S.N.F.	Fat	S.N.F.	Fat	S.N.F.	Fat	S.N.F.	Fat	S.N.F.	
a.m.	2.7	8.37	3.0	8.58	3.0	8.61	2.3	8.14	3.2	8.47	3.8	8.33	4.7	8.98	3.7	8.31	3.35	8.38	2.9	8.40	
Dried grass	3.2	8.77	4.35	8.63	3.4	8.74	3.3	8.14	3.7	8.78	3.5	8.49	5.2	9.05	4.4	8.65	4.0	8.61	4.0	8.55	
Control	a.m.	2.9	8.40	2.6	8.48	2.9	8.41	2.6	8.06	3.1	8.44	3.9	8.30	5.0	8.83	4.0	8.37	3.1	8.39	3.35	8.62
a.m.	3.3	8.70	3.9	8.57	3.25	8.71	2.7	8.30	3.5	8.76	3.8	8.53	4.9	9.24	4.5	8.66	3.5	8.72	3.8	8.77	
Dried grass	a.m.	3.1	8.66	2.7	8.81	3.4	8.78	2.7	8.30	3.25	8.60	3.7	8.55	5.5	9.07	3.9	8.55	3.6	8.67	3.4	8.69
Control	a.m.	3.2	8.91	4.1	8.86	3.2	8.93	3.0	8.41	3.5	8.72	3.6	8.72	5.3	9.2	4.7	8.75	3.8	8.82	3.9	9.09
a.m.	3.0	8.83	3.0	8.87	3.2	8.85	2.9	8.62	3.2	8.68	3.7	8.45	5.05	9.01	4.1	8.53	3.5	8.91	3.2	9.10	
p.m.	3.5	9.10	4.2	9.13	3.3	9.17	3.2	8.66	3.4	8.91	3.7	8.70	5.25	9.2	4.7	8.64	3.7	9.19	3.6	9.27	
Group B																					
	Queenie		Kathy 3		Dairymaid		Fancy		Grace		Ruth		Eva		Lottie		Cherry		Gladys		
	Fat	S.N.F.	Fat	S.N.F.	Fat	S.N.F.	Fat	S.N.F.	Fat	S.N.F.	Fat	S.N.F.	Fat	S.N.F.	Fat	S.N.F.	Fat	S.N.F.	Fat	S.N.F.	
a.m.	3.0	8.37	2.65	8.14	2.85	8.53	2.7	7.80	3.3	7.86	2.7	8.31	3.75	8.82	3.7	8.49	3.5	8.76	3.1	8.64	
Control	p.m.	4.2	8.66	3.6	8.49	3.3	8.72	3.3	8.17	3.8	8.46	2.8	8.52	4.9	9.19	4.6	8.74	4.7	9.02	4.4	8.83
Dried grass	a.m.	3.5	8.52	2.9	8.12	2.8	8.33	3.0	7.93	3.1	8.35	2.7	8.25	4.4	9.08	3.8	8.53	4.0	8.82	3.1	8.44
Control	p.m.	4.2	8.86	3.4	8.41	3.1	8.64	3.1	8.14	3.1	8.64	3.0	8.41	4.8	9.25	4.4	8.88	4.7	9.27	3.7	8.72
a.m.	3.7	8.78	3.1	8.39	2.8	8.58	3.2	8.35	3.2	8.66	2.5	8.45	4.6	9.19	4.1	8.84	4.3	9.12	3.2	8.71	
Control	p.m.	4.1	9.06	3.5	8.74	3.1	8.94	3.25	8.61	3.3	8.91	3.2	8.40	4.9	9.29	4.3	9.07	4.8	9.42	3.7	8.97
a.m.	3.5	8.76	3.2	8.65	2.85	8.50	3.1	8.39	3.2	8.68	2.65	8.57	4.4	9.15	4.0	9.00	4.3	9.34	3.55	8.97	
Dried grass	p.m.	4.0	9.12	3.7	8.82	3.05	9.0	3.4	8.74	3.2	8.85	3.1	8.69	4.7	9.45	4.4	9.15	4.9	9.69	4.2	9.29

the usual way from the specific gravity of the milk. The figures are given in Table XII as averages of each 5-week period.

An analysis of the figures for fat content was made by Mr Bartlett on the same lines as the analysis of the milk yields.

"Thus ten values  $yF$ , representing the effect on fat content of Dried grass *v.* Control, were obtained. Separate values were calculated for the morning and evening milks, and these two values combined without weighting.

"The following table gives the result of this analysis:

Table XIII. *Summary of statistical analysis of butterfat percentage figures*

	Dried grass <i>v.</i> Control	S.E.	Sig. diff. from 0
Fat content	+0.016	0.023	0.052
% general mean	+0.44	0.64	1.44

"The effect of Dried grass *v.* Control on the fat content of the milk is quite insignificant.

"This conclusion would not be affected by a more accurate weighting of the fat content figures for the morning and evening milk yields. For example, supposing the correct weighting on the average should be 2:1 instead of 1:1. The loss of information would then be 1/9 of the information available; this means that, by a more accurate weighting, we might expect to increase the ratio of the observed effect to its standard error from 0.70 to 0.74. The analysis given here is therefore sufficient."

The figures for solids-not-fat were treated in the same way as the butterfat values with the following result:

Table XIV. *Summary of statistical analysis of solids-not-fat percentage figures*

	Dried grass <i>v.</i> Control	S.E.	Sig. diff. from 0
Solids-not-fat	+0.0091	0.0165	0.0368
% general mean	+0.105	0.190	0.423

As was the case with the fat content, the effect of Dried grass *v.* Control on the content of solids-not-fat of the milk is quite insignificant.

It is obvious, therefore, that the inclusion of dried grass in the ration has not affected the quality of the milk in so far as the content of fat and solids-not-fat are concerned.

## COLOUR OF THE BUTTERFAT

The secondary issue, the effect of the two rations on the colour of the butterfat in the milk of the different breeds, was also examined.

The yellow colour of the butterfat of each cow was measured at the end of each 5-week period, using a Lovibond tintometer. This gives a quantitative value to the colour of the fat.

Before considering the yellow colour values of the butter, it is of interest to note the carotene content of the foodstuffs used. These are summarised in Table XV.

Table XV. *Figures showing carotene content of foodstuffs\**

Dried grass	Cabbage	Kale	Hay
20.5	15.4	14.0	1.6
20.3	—	13.4	1.8
21.8	—	—	—
22.1	—	—	—
20.5	—	—	—
19.5	—	—	—
20.8	—	—	—
16.8	—	—	—

\* Stated as mg. per cent. of the dry matter.

The values for dried grass are lower than is usual for artificially dried grassland herbage, but much superior to those for the hay. Cabbage and kale are rich in carotene, but were never given in sufficient quantity to affect the colour of the milk.

Using the values determined on the foodstuffs, it is possible to calculate the actual carotene contents of the control rations and the dried grass rations.

Table XVI. *Amount of carotene fed to control and experimental groups in mg. per head per day*

	First period			Second, third and fourth periods		
	Control	Experimental		Control	Experimental	
		Guernseys	Other cows		Guernseys	Other cows
Hay	93.5	93.5	93.5	118.5	118.5	118.5
Kale, cabbage	295.7	295.7	295.7	—	—	—
Dried grass	—	858.0	686.4	—	823.6	658.9
Total	389.2	1247.2	1075.6	118.5	942.1	777.4

During the first period the use of kale and cabbage raised the carotene intake above that of the other three periods, but throughout all four

periods the carotene intake on the dried grass rations was considerably greater than on the control diet.

The yellow colour was determined on individual samples taken in the fifth week of each period. The reason for sampling at this time was to allow the ration to exert the greatest effect of which it was capable in the somewhat short periods employed. It may be said that no one of the colour values reached the level which has been attained in previous experiments when a carotene-rich diet was fed throughout the whole of the winter.

In order to get some idea of the rate at which the colour was developed, samples were taken at the end of the third week in the third and fourth periods, and in the fourth week of the second period.

Table XVII. *Total yellow colour of the butter from the individual cows*

		First period	Second period		Third period		Fourth period	
		5th week	4th week	5th week	3rd week	5th week	3rd week	5th week
Breed	Cow	Dried grass	Control		Dried grass		Control	
Group A	Shorthorn Betty	4.8	—	2.4	3.2	4.0	2.8	2.0
	Hollyhock	4.0	3.6	2.4	2.8	3.6	3.2	1.6
	Friesian Pearl	5.6	—	3.2	3.6	4.8	4.0	2.8
	Saucy	5.6	3.2	4.0	4.8	6.0	4.4	2.8
	Ayrshire Ninna	2.8	2.4	2.4	2.8	3.6	2.4	1.2
	Tiny	7.6	—	3.6	4.8	5.6	3.2	2.0
	Guernsey Nellie	10.8	6.0	5.0	9.6	10.8	5.2	4.0
	Bessie	6.8	—	4.0	5.2	5.6	3.2	2.8
	Shorthorn Olive	8.4	—	2.8	3.6	4.4	2.8	2.0
	Martha	6.8	4.8	3.2	3.6	3.2	2.8	2.0
Group B	Control		Dried grass		Control		Dried grass	
	Shorthorn Queenie	4.0	6.8	6.4	3.2	2.8	4.4	4.8
	Kathy 3	2.4	—	4.4	2.4	1.6	2.8	3.2
	Friesian Dairymaid	2.0	2.4	3.6	2.0	1.6	3.6	3.6
	Fancy	2.0	—	5.2	3.2	2.4	3.2	3.6
	Ayrshire Grace	3.2	—	3.6	2.8	2.8	3.6	3.6
	Ruth	4.4	6.4	5.6	3.6	3.2	4.8	4.8
	Guernsey Eva	6.4	10.4	11.2	6.4	5.2	8.4	10.4
	Lottie	9.6	—	16.4	7.2	5.6	9.2	13.6
	Shorthorn Cherry	2.0	—	2.8	2.0	1.6	2.4	2.4
	Gladys	5.2	9.2	8.8	4.8	3.2	4.4	6.0

These figures are all intermediate between the preceding and the succeeding values. Examination of the figures obtained shows clearly that the experimental technique adopted was not suited to an exact quantitative study of the effect of extra carotene, at the level of intake

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obtaining in this experiment, on the yellow colour of the butter, owing to the lag in response to change of carotene ration. The 5-week period was probably not sufficient for the full effect to be observed, and the change-over in treatments made the body reserve of carotene in the cow an indefinite quantity. Where the level of carotene intake is high a clear-cut difference can be obtained. In two trials (10) carried out with South Devon cows and Shorthorn cows the following results were obtained when 40 lb. of A.I.V. fodder (silage made with added acid) were fed per head daily.

Table XVIII. *Total yellow colour of the butter produced by two groups of cows on a normal winter ration and on one containing 40 lb. of A.I.V. fodder (stated in Lovibond units)*

	Period 1 Control	Period 2 A.I.V. fodder	Period 3 Control
South Devon cows	4.0	8.4	2.8
Shorthorn cows	2.0	8.4	2.4

The 40 lb. of A.I.V. fodder supplied about 1800 mg. of carotene daily.

The periods were each of 5 weeks' duration, the butter samples being taken in the last week of each period.

In the present experiment no statistical analysis is, of course, needed to establish the qualitative effect of the extra carotene in the dried grass ration. The unanimous increase in yellow colour from the end of a "control period" to the end of a "dried grass period", associated with the unanimous decrease at the same time from the "dried grass period" to the "control period" for cows of the other group, is quite sufficient to establish the reality of this effect.

There is also quite definitely a tendency for the yellow colour to decrease with time; this effect being seen by comparison of, say, the second period with the fourth period, for which the cows received the same ration. The same effect is noticeable with the first and third periods, but this is hardly a fair comparison, since the carotene content of the ration for the first period was higher than the third. This decrease is presumably due to a gradual depletion of the carotene body reserve of the cow during the control periods.

### STATISTICAL ANALYSIS OF RESULTS

"It was thought to be of value to make a straightforward analysis of variance of the yellow colour variation. Several limitations of this analysis must be noted. In the assessment of residual variation after the treatment effect has been eliminated, no account has been taken of

the decrease with time noted above. Nor in measuring the response to carotene have the anomalous rations, such as the higher carotene ration given during the first period, or the higher ration given to the Guernsey breed, been considered separately. Further, the character of the variation is not suited to exact tests of significance, the variation becoming progressively greater as the yellow colour level rises. This latter fact causes cows with high yellow colour butter to show corresponding high responses to extra carotene, but this does not necessarily imply a correspondence between the carotene level of the butter and response in carotene content to extra carotene in the ration, owing to the non-linear relationship between yellow colour and carotene content. A more comprehensive analysis might have been made, but in view of the technique of the experiment any conclusions would still be limited in scope. The analysis made, in spite of its limitations, can still be regarded as summarising the variation in yellow colour that was actually obtained.

## ANALYSIS OF VARIANCE

"The four readings per cow corresponding to the end of each experimental period were considered; the carotene effect being measured, for example, by taking the difference between the yellow colour for the dried grass periods and for the control periods. The division of the cows into two groups, with different times for the same ration, was ignored.

Table XIX

Factor	D.F.	Sums of squares	Variance
Residual variation per cow between periods	40	48.42	1.21
Carotene effect:			
Mean	1	155.12	155.12
Differences between breeds	3	34.21	11.40
Difference between cows of same breed	16	24.16	1.51
Variation of average level:			
Between breeds	3	236.03	78.68
Between cows of same breed	16	143.74	8.98
Total	79	641.68	

"The mean treatment effect 155.12 may legitimately be compared with the residual variance 1.21. This mean effect accounts for the major part of the variation due to change of treatment, though the difference in response to ration is still greater between than among breeds. Similarly, the variation of the average yellow colour per cow is greater between than among breeds. This is due entirely to the inclusion of the Guernsey breed, as can be seen from the following supplementary table.

Table XX

Variation between breeds	D.F.	Sums of squares	Variance
Carotene effect:			
Guernsey <i>v.</i> Remainder	1	33.54	33.54
Among other breeds	2	0.69	0.35
Average level:			
Guernsey <i>v.</i> Remainder	1	235.98	235.98
Among other breeds	2	0.05	0.03

"It should be remembered that the superiority of the Guernsey—apart from possible chance variation which has not been tested here statistically—will be due in part at least to the higher level of carotene in the ration given to this breed. There is no evidence of differences among the other breeds, the variation being in fact much below expectation."

The most important point which has arisen out of the examination of the values for yellow colour of the butterfat in the milk is the effect of the inclusion of dried grass in the ration. This has invariably resulted in an increase in depth of yellow colour. The superiority of the Guernsey in depth of yellow colour in the milk, irrespective of the ration, is also evident. Here too the inclusion of dried grass resulted in an increase in yellow colour of the fat in the milk. The difference which had previously been noted (10) between the response in yellow colour of cows of the Shorthorn and Ayrshire breeds to inclusion of dried grass in the ration was not obtained under the conditions of this experiment. This was undoubtedly due to the short periods, and the experiment has not given a full answer to the question for which it was designed. Where response in colour of the milk fat is sought, it is essential to feed the carotene-rich ration over a long period. The dairy cow does, however, respond in some measure to differences in the carotene content of the ration.

#### WEIGHT OF COWS

The cows were weighed at the beginning and end of each period, and the weights are given in Table XXI. The weights are all the average of three consecutive days' weights.

The statistical analysis of the figures for the increase or decrease in weight of the cows during each 5-week period gave the results summarised in Table XXII.

The inclusion of dried grass has been beneficial in regard to the effect on the weights of the cows, this difference being significant.

Table XXI. *Weights of cows in lb.*

Cow	Dec. 8	Jan. 12	Feb. 16	Mar. 23	Apr. 27	
Group A	End of ...	Dried grass period	Control period	Dried grass period	Control period	Average
Betty	1094	1050	1062	1078	1087	1074
Hollyhook	1078	1031	1052	1064	1064	1058
Pearl	1302	1272	1288	1281	1318	1292
Saucy	1276	1283	1311	1295	1262	1285
Ninna	1134	1123	1110	1115	1087	1114
Tiny	1064	1031	1015	1036	1064	1042
Nellie	966	929	936	971	973	955
Bessie	1090	1089	1085	1092	1113	1094
Olive	1050	1043	1055	1062	1080	1058
Martha	—	1041	1080	1085	1080	1072

Group B	End of ...	Control period	Dried grass period	Control period	Dried grass period	
Queenie	936	884	947	957	989	943
Kathy 3	1204	1174	1216	1225	1253	1214
Dairymaid	1230	1211	1230	1227	1227	1225
Fancy	1024	1036	1062	1064	1103	1038
Grace	—	1073	1043	1055	1071	1061
Ruth	1197	1185	1213	1216	1255	1213
Eva	901	891	922	919	933	913
Lottie	955	940	966	959	987	961
Cherry	945	978	962	959	980	965
Gladys	1013	975	1010	994	994	995

Table XXII. *Average superiority of dried grass per 5-week period on weight (lb.) of cow*

Dried grass v. Control	S.E.	Sig. diff. from 0
+ 8.58	3.07	6.84

## CONDITION OF THE COWS

Constant observation of the cows showed that their condition, as judged from their appearance, was good throughout the whole of the experiment. While on the dried grass ration they seemed to improve somewhat in condition, which is borne out by the figures in Table XXII, giving the analysis of the changes in weight during each period, and at the end of the experiment all the cows appeared to have improved in general condition.

The droppings throughout were normal and no evidence of undue looseness or costiveness was observed while the cows were on the ration containing artificially dried grass.

## SUMMARY

An experiment was carried out with two groups of ten cows each, made up of two Guernseys, two Ayrshires, two Friesians and four Shorthorns.



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The experiment was of the change-over type, the experimental period of 20 weeks being subdivided into four periods of 5 weeks, each cow alternating between the two treatments.

In two of the periods a normal winter ration of roots, hay and concentrates was fed. In the other two periods artificially dried grass replaced a proportion of the concentrates, an average of 8 lb. being fed per head daily. The two types of ration provided equal amounts of starch equivalent and protein equivalent, but the carotene intake was greater in the "dried grass ration".

A statistical analysis of the difference in milk yields due to the contrast "Dried grass" *v.* "Control" revealed no signs of any effect, and if any actual effect does exist, it is quite negligible for the 5-week periods of this experiment.

A similar analysis on the fat content and solids-not-fat figures revealed no sign of change in fat or solids-not-fat content due to treatment.

An analysis of the butter yellow-colour figures was made, though it is pointed out that there are limitations to the interpretation of this analysis. The response to carotene in the ration is, however, quite obvious; it is most marked for the Guernsey breed.

The condition of the cows remained good throughout the duration of the experiment, but they appeared to do better on the ration including dried grass, which showed a significant advantage over the ordinary winter ration in respect of weight increases.

It is considered essential to increase the length of the periods in experiments of this type, more particularly when the effect of the ration on the colour of the fat in the milk is to be investigated.

The quality of artificially dried grass might have been better, but that used was not far different from the average type of material which might come on the market.

### ACKNOWLEDGMENTS

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# APPENDIX I

## Detailed yields in lb. of individual cows

Week ending	Group A										Total	Average
	Shorthorn		Friesian		Ayrshire		Guernsey		Shorthorn			
	Betty	Hollyhook	Pearl	Saucy	Ninna	Tiny	Nellie	Bessie	Olive	Martha		
Dec. 17	232½	328½	289	241½	414	207	232½	257½	203½	250½	2656½	265.7
" 24	219½	311½	283½	228½	409	203½	218	247½	204½	240½	2565½	256.6
" 31	218	331½	280½	221	415½	200½	212	227½	205½	221½	2534½	253.5
Jan. 7	215½	333½	275½	218	413½	199	207	220	201½	216½	2500½	250.0
" 14	204	333	269½	212½	408½	191½	203½	197	172	213½	2403½	240.3
"	1089½	1638	1397½	1121½	2058½	1001½	1073½	1150	987½	1142½	12660½	1266.1
"	217.9	327.6	279.5	224.3	411.8	200.3	214.8	230.0	197.5	228.5	2532.1	253.2
Second period control ration												
Jan. 21	204½	339½	261½	200½	397	197½	197	190	182½	201½	2371½	237.3
Jan. 28	209½	317½	250	250½	386½	189½	186½	183½	187½	200½	2315½	231.6
" Feb. 4	208½	325½	248	199½	390½	190½	184½	174½	187	204	2312½	231.2
" 11	207	335½	248½	203½	390	190	180	184½	190½	202	2330½	233.1
" 18	201½	334½	246½	197½	388	193½	185	180½	182½	187½	2297½	229.7
"	1031	1651½	1254½	1006½	1952½	961½	933	912½	929½	995½	11627½	1162.7
"	106.2	330.4	250.9	201.3	390.5	192.3	186.6	182.5	185.9	199.1	2325.5	232.6
Third period dried grass												
Feb. 25	197½	329	247½	191½	372½	193	181½	166½	180½	182½	2241½	224.2
Mar. 4	189½	322½	246	187	357½	181½	181½	165½	174½	181½	2186	218.6
" 11	189	322½	233½	183	360½	179½	177½	164½	162½	184½	2156½	215.7
" 18	178½	326½	213	182½	346	182½	161½	161½	179	183½	2114½	211.5
" 25	170½	313½	191½	180½	337½	180	175	151½	178½	186½	2064	206.4
"	925	1614½	1131½	923½	1774½	916	876½	809	874	918½	10763	1076.3
"	185.0	322.9	226.3	184.8	354.9	183.2	175.3	161.8	174.8	183.7	2152.6	215.3
Fourth period control ration												
Apr. 1	164½	301½	198	179	318½	177	169½	148½	173½	184½	2014½	201.5
" 8	159	305½	199	170	302	173½	171½	135½	165½	172½	1851½	185.2
" 15	156½	281½	191½	172½	291½	172½	174½	126½	159	165½	1892½	189.2
" 22	149½	287	194½	165½	290½	164½	172½	127½	161½	169½	1883	188.3
" 29	141½	276½	192½	170½	294½	170½	164½	124	158½	169½	1863	186.3
"	770½	1452	976	857½	1497½	858	852½	660½	818	862	9604½	960.5
"	154.1	280.4	195.2	171.5	399.5	171.6	170.6	132.1	163.6	172.4	1920.9	192.1

Group B

Week ending	Group B										Total	Average
	Shorthorn		Friesian		Ayrshire		Guernsey		Shorthorn			
	Queenie	Kathy 3	Dairymaid	Fancy	Grace	Ruth	Eva	Lottie	Cherry	Gladys		
Dec. 17	205½	296	353½	306½	211	207½	234	217	187	242½	2461	246-1
" 24	194½	291½	333½	297	215	206½	230	230½	192½	231	2422	242-2
" 31	198½	294½	331	274½	209	196½	219½	228½	196½	238½	2386½	238-6
Jan. 7	187½	286½	328	269½	200	189½	212½	222½	185½	241½	2322½	232-3
" 14	170½	276½	312½	270½	197½	186½	203	215½	176½	239	2248	224-8
" 21	957½	1444½	1658	1418½	1032½	986½	1099	1113½	937½	1192½	11839½	1184-0
" 28	191-5	289-0	331-6	283-7	206-5	197-3	219-8	222-8	187-6	238-5	2368-0	236-8
Jan. 21	176	215½	298½	273	219	181½	203	200½	173½	230½	2170½	217-1
" 28	181	247	281½	271½	219½	171	204	200½	168	229½	2174	217-4
Feb. 4	181½	243½	279½	274	207½	172½	197½	196½	167	225½	2145½	214-6
" 11	176½	246½	280½	276½	204½	168½	195½	191½	166	223½	2129½	212-9
" 18	175½	237½	273½	265½	207½	157½	189½	184½	159½	203½	2054½	205-5
" 25	890½	1189½	1414½	1360½	1058	850½	989½	973½	834½	1112½	10674½	1067-5
" 28	178-2	238-0	282-9	272-2	211-6	170-2	198-0	194-7	166-9	222-6	2135-0	213-5
Feb. 25	170½	215½	274½	271	195½	156½	188½	180½	160½	212½	2025½	202-6
Mar. 4	171½	212½	267½	268½	183½	151½	193½	184½	157½	207½	1998½	199-8
" 11	172½	215½	258½	265	183½	147½	190½	183½	161	206½	1983½	198-4
" 18	169	212	240½	245½	176½	141	184	177½	156½	203	1905½	190-5
" 25	166½	214½	253½	257	181½	140	187½	182½	159½	203½	1945	194-5
" 28	850	1069½	1294½	1306½	920½	737½	944½	908½	795	1032½	9858	985-8
" 29	170-0	214-0	258-9	261-3	184-1	147-5	188-9	181-7	159-0	206-5	1971-6	197-2
Apr. 1	158½	205½	255½	257½	175	136	182½	177	148½	191½	1887½	188-8
" 8	160½	206½	253½	221	151½	133½	151½	169½	144½	178½	1769½	177-0
" 15	163½	201½	249½	179	159½	129½	167½	171	151½	177½	1749	174-9
" 22	161	204	256½	223½	163½	128	167½	171½	151	177½	1804½	180-5
" 29	157½	205	247½	229½	159½	126½	168½	162½	150½	176½	1784	178-4
" 30	801½	1022	1262½	1110½	808½	653½	837½	851½	745½	902	8994½	899-5
" 31	160-3	204-4	252-5	222-1	161-7	130-7	167-5	170-3	149-2	180-4	1798-9	179-9

# THE COMPOSITION AND NUTRITIVE VALUE OF MARROW STEM KALE AND THOUSAND HEAD KALE

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## INTRODUCTION

IN pursuance of a scheme of research designed to secure information, from the nutritional standpoint, about the crops that are customarily fed to live stock in the green condition, the writers recently turned their attention to the study of the nutritional characteristics of those two important winter forage crops, marrow stem kale and thousand head kale. Writing in 1927, T. B. Wood<sup>(1)</sup> stated that, among the crops on which sheep are folded in the winter, marrow stem kale was coming into continually increasing prominence on account of its great power of resisting drought on light land. He pointed out that Kellner gave no figures for marrow stem kale, which was not known at the time when this German authority was carrying out his classical investigations. The figures in "Rations for Live-Stock"<sup>(2)</sup>, namely, 14·3 per cent. of dry matter and 8·9 per cent. of starch equivalent, were worked out, according to Kellner's formula, from a small number of analyses and assumed digestion coefficients. For so important a crop they stood in urgent need of revision.

In a more recent publication, dealing with the composition and uses of home-grown feeding stuffs, H. E. Woodman<sup>(3)</sup> places special emphasis on the reliability and drought-resisting character of marrow stem kale, which is capable of producing, at a relatively low cost, a heavy yield of nutritious and succulent food. Yields of 20 tons or more per acre are by no means uncommon. One acre will provide green food for fifteen cows until after Christmas, at which time it is usual to go on to swedes

or mangolds. This change-over, however, is not actually necessary in the south, since marrow stem kale is able to withstand the cold of an average winter. On many farms, marrow stem kale is preferred to cabbages and roots. For sheep folding, it has an advantage over roots in that it stands up well from the ground, and the edible portions are therefore always clean. It is also suitable for carting off and feeding in yards or on grass. Good results have been obtained with dairy cows when the animals have been receiving as much as 125 lb. of the kale per head per day. Though not yielding so heavily as the marrow stem kale, the thousand head variety, on account of its winter hardiness, is able to administer to the needs of stock during late winter and early spring, thus helping very materially to keep up the farmer's supply of fresh green food throughout the whole twelve months. Ewes, after lambing, may be drafted from the lambing pens into folds on thousand head kale, or the kale may be cut and fed on grass.

The potentialities of marrow stem kale from the standpoint of yield are brought out strikingly by the results of recent manurial trials at Rothamsted. Commenting on the results of these trials, Sir E. J. Russell<sup>(4)</sup> writes that: "The kale responded well to nitrochalk even up to 6 cwt. per acre and gave the remarkable yield of over 38 tons per acre when left unthinned."

Marrow stem kale is apparently growing in favour on the Continent and has formed the subject of several recent reports. This fact is revealed by C. Crowther<sup>(5, 6)</sup> in his reviews of animal nutrition research carried out in 1932 and 1933. In comprehensive tests at the Swedish Central Experiment Station, the composition and digestibility of the green crop at different stages of growth, and of silage made from it, have been studied. Similar investigations have also been carried out by German authorities, and reference to the findings of these continental researches will be made during the discussion of the results of the present investigation.

Sufficient has been written in this short introduction to emphasise the need for investigations into the composition and nutritive value of the two varieties of kale as grown in this country. If the nutritive characteristics of these winter forage crops can be influenced to any marked extent by climatic factors, it by no means follows that the results of trials in Germany and Sweden, with their characteristically severe continental winters, can be taken as representing the full possibilities of these crops when grown for utilisation during the milder and more open winters usually experienced in this country.

## SCOPE OF PRESENT INVESTIGATION

The marrow stem kale and thousand head kale were grown for the purpose of the present trials on adjacent  $\frac{1}{2}$ -acre plots situated on the gravel-loam soil of the Howe Hill University Farm at Cambridge. The date of sowing was May 2, 1934, the seed being drilled at the rate of 6 lb. per acre in rows 18 in. apart. Manurial treatment was restricted to an application of sulphate of ammonia (2 cwt. per acre), but it should be pointed out that marrow stem kale had been grown on this area during the previous season, when the land received, per acre, 4 cwt. of superphosphate of lime, 1 cwt. of sulphate of potash and 2 cwt. of sulphate of ammonia.

In order to throw light on the effect of singling-out on composition and nutritive value, several long rows were submitted to this operation. An examination of this singled-out area during the winter of the experiment revealed the fact that the spacing between the plants was quite irregular, varying from as little as 9 in. to as much as 30 in., with an average spacing of 18 in.

Owing to the prevalence of hot, dry weather, neither crop of kale made very good progress up to the end of July. An excellent response was shown, however, to the rains of August and September, so that at the time of the beginning of the feeding trials in mid-September, both kale crops presented a green, healthy appearance. Only a very small proportion of brown leaves was to be seen, but it should be noted that throughout the feeding trials, the whole plants, including any wilted leaves, were fed to the digestion-sheep, since such brown leaves would be eaten by sheep when folded on kale.

Despite the satisfactory appearance of the crops, the yield of kale in both cases was by no means heavy. Yield tests on a large number of small areas served mainly to emphasise the difficulty of estimating the yield of crops displaying such lack of uniform growth. Over the main experimental area of unthinned marrow stem kale, the yield per acre of green crop varied from 13.8 tons (4249 lb. dry matter) to 15.7 tons (4897 lb. dry matter). The corresponding variation for the singled-out marrow stem kale was from 10 tons of green crop per acre (2981 lb. dry matter) to 12.6 tons (3931 lb. dry matter), while in the case of the thousand head kale, the range of variation was from 9.9 tons (3537 lb. dry matter) to 11.8 tons (4427 lb. dry matter). It is not desired to read any significance into these yield comparisons, since if such a purpose

had entered into the plan of the present investigation, a very different experimental lay-out and procedure would have been necessary.

The weather during the winter of 1934-5 was, on the whole, mild and open, with an abundance of rain. The first night frosts occurred at the end of October and beginning of November. During the rest of November, however, there was very little frost, while December was characterised by extraordinary mildness and wetness. The January of 1935 was a much colder month, the later part (January 27-30 inclusive) being given over to snow and frost. During February, apart from a few slight night frosts, the weather was again mild and wet. Night frosts were encountered on the following dates during the period of the experiment: October 30, 31; November 1, 2, 8, 13, 14, 20; December 17, 22; January 7, 8, 9, 10, 11, 12, 13, 16, 27, 28, 29, 30; February 6, 7, 8, 9, 10, 23, 24, 26, 27. It may be noted that the kale crops stood the winter remarkably well, and the plants remaining after the completion of the trials at the end of February were still green and healthy.

Since it appeared probable that the nutritive properties of the kales might undergo continuous deterioration with the progress of the winter season, it was decided to make determinations of composition and digestibility both before and after the New Year. The main work, therefore, was carried out according to the following programme:

	Period of investigation
Marrow stem kale (unthinned)	Sept. 14-Oct. 9, 1934
Marrow stem kale (singled-out)	Oct. 10-Nov. 3, 1934.
Thousand head kale	Nov. 4-Nov. 26, 1934
Marrow stem kale (unthinned)	Jan. 1-Feb. 4, 1935
Marrow stem kale (singled-out)	Jan. 22-Feb. 11, 1935
Thousand head kale	Feb. 12-Mar. 4, 1935

#### COMPOSITION OF MARROW STEM KALE (UNTHINNED), MARROW STEM KALE (SINGLED OUT) AND THOUSAND HEAD KALE

*Dry matter content.* In large samples taken day by day over the period September 14 to October 9, the range of variation in dry matter content of the unthinned marrow stem kale was from 11.4 to 14.8 per cent., with a mean value of 13.3 per cent. Part of this daily variation was no doubt due to variability in the weather conditions at the times of cutting, but the effect of weather was reduced to a minimum by spreading out the kale samples overnight on a cold stone floor before proceeding to the dry matter determinations. In this way, most of the superficial moisture (if any) disappeared. Tests carried out on samples that were free, at the time of cutting, from superficial moisture showed



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that such overnight storage at this time of the year led to no significant reduction of moisture content. The figures given above may be taken as affording a fairly reliable guide to the dry-matter content of unthinned marrow stem kale in the autumn.

The daily determinations made at a later date (January 1 to February 4) suggest that unthinned marrow stem kale tends to become slightly richer in dry matter with the progress of winter. Over this period the percentages of dry matter ranged from 13.1 to 16.3 per cent., with a mean value of 14.2 per cent., compared with 13.3 per cent. for the September-October period. These values show quite good agreement with those recorded by Edin and co-workers<sup>(7)</sup>, who, on the basis of investigations in Sweden, Denmark and Finland, give dry-matter percentages for marrow stem kale varying from 13.8 to 14.8 per cent. In German investigations, Bünger and co-workers<sup>(8)</sup> found their experimental crop to contain, on the average, 13.1 per cent. of dry matter, while Schmidt and co-workers<sup>(9)</sup> noted distinctly higher percentages of dry matter in marrow stem kale during November than in September. Wohlbier<sup>(10)</sup> found much greater variation in the dry-matter content than would be expected from the results of the present investigation, values as wide as 7.3 and 17.5 per cent. being quoted.

The data for the singled-out marrow stem kale were very similar to those for the unthinned crop, the dry-matter percentages for the period October 10 to November 3 varying from 12.0 to 16.0 per cent., with a mean value of 13.5 per cent., while those for the period January 22 to February 11 ranged from 12.4 to 16.2 per cent., with an average value of 14.2 per cent. It would appear, therefore, that singling out had exercised little effect on the dry-matter content of the kale.

The values for the thousand head kale were uniformly higher than those for the marrow stem variety. From November 4 to 26, the daily dry-matter determinations gave results varying from 14.4 to 17.4 per cent. (mean value = 15.7 per cent.) and from February 12 to March 4, the range of variation was from 14.2 to 17.3 per cent. (mean value = 16.0 per cent.). It may be concluded, therefore, that thousand head kale is somewhat richer in dry matter than marrow stem kale, a finding consistent with the fact that the latter has arisen by crossing thousand head kale with the moister and more succulent kohlrabi (12.7 per cent. of dry matter).

*Nature of the marrow.* The stem in marrow stem kale is swollen as in kohlrabi, but is elongated instead of spherical, thus giving rise to a form intermediate between kohlrabi and thousand head kale. Since

the feeding value of the stem is mainly determined by its content of succulent marrow, it was considered desirable to carry out an investigation of the chemical nature of this material.

The leaves were removed from a number of plants of the singled-out crop (stems varying from  $1\frac{1}{2}$  to  $2\frac{1}{2}$  in. thickness), and the marrow was obtained by stripping the rind from the stems, the rind being taken as including not only the outer skin, but also the layers leading up to and including the tough woody xylem. The marrow consisted, therefore, of the innermost soft and pale green tissue lying within the xylem. It was first put through a mincer, stirred up with a little distilled water and strained through linen, the juice and small amount of insoluble residue being investigated separately.

Tests on juice: (1) direct reduction with Fehling's reagent showed the presence of a large amount of reducing sugar; (2) iodine test showed starch to be absent; (3) alkaline sodium hypobromite caused copious evolution of nitrogen, suggesting presence of an appreciable amount of asparagine; (4) boiling gave rise to coagulation of a small amount of protein (confirmed by Millon's reaction); (5) carbazole and diphenylamine reactions showed absence of nitrate.

Tests on insoluble residue: (1) negative test for pentosans by aniline acetate reaction; (2) negative test for pectose by extraction with 0.5 per cent. ammonium oxalate; (3) negative test for starch by iodine reaction; (4) residue was probably composed mainly of cellulose and allied compounds (see Table VI for percentage of fibre in the marrow).

*Sugar content of marrow.* For the purpose of quantitative determinations of sucrose and reducing sugar, the marrow from a number of swollen stems was reduced to a pulp and a 10-gm. portion was weighed and washed into a 500 c.c. graduated flask with distilled water. The determination was carried out in duplicate. Deproteination was effected by slowly running in 3-4 c.c. of 5 per cent. dialysed iron, the volume being then made up to the 500-c.c. mark with distilled water and a few drops of ether to subdue frothing. The contents of the flask were quickly shaken and, after settlement of the flocculent precipitate, filtered through a dry filter.

20 c.c. of the water-clear filtrate was used for determining the reducing sugar by Bertrand's method. For the estimation of total sugar, 100 c.c. of the filtrate was heated in a water-bath at 70-80° C. for 20 min. with the addition of 10 c.c. concentrated HCl. After cooling and making the liquid almost alkaline with solid  $\text{Na}_2\text{CO}_3$ , the volume was measured (in order to make allowance for the slight volume change

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during the operations), and 20 c.c. portions were pipetted into Erlenmeyer flasks for making the determination in duplicate.

In Table I are given results for the dry-matter and sugar content of marrow from both thick and thin stems of marrow stem kale cut in the first half of October, and for thick stems cut in the following January.

Table I. *Dry-matter and sugar content of marrow  
from marrow stem kale*

Date of sampling	Oct. 12, 1934	Oct. 14, 1934	Jan. 24, 1935
Thickness of stems (in.)	1½-2½	½-1	1½-2½
Dry matter (%)	9.40	11.40	10.61
On basis of dry matter:			
Reducing sugar (%)	40.96	50.32	21.85
Sucrose (%)	10.91	4.05	23.68
Total sugar (%)	51.87	54.37	45.53

The determination carried out on October 12 shows that the marrow from the thick stems of the crop contained, at this date, less than 10 per cent. of dry matter, of which rather more than half was composed of a mixture of invert sugar and sucrose in the proportion of about 4 to 1. The data in Table VI reveal the fact that the marrow contained, on the basis of dry matter, 58.4 per cent. of N-free extractives, from which it may be concluded that nearly the whole of the N-free extractives in the marrow is in the form of sugar.

It was thought possible that in cases where the stems of the crop had not undergone the characteristic swelling, the marrow from such undeveloped stems might have a different character from that in the swollen stems. That this was not the case, however, is shown by the figures in Table I for the analysis of the marrow from stems of ½-1 in. diameter. Apart from the rather higher content of dry matter and a much higher proportion of invert sugar to sucrose, the general character of the marrow was similar to that from the more developed stems.

The results in Table I for the marrow examined at a later date in the winter (January 24) are of particular interest, since although the percentage of total sugar was still remarkably high (45.5 per cent. on the basis of dry matter) the distribution between sucrose and invert sugar was very different from what had been found in the previous October, the sucrose (23.7 per cent. of the dry matter) now being present in slightly higher concentration than the reducing sugar (21.9 per cent.). Whether this change could be attributed to the influence of the frosts of January cannot be stated with certainty. At this stage it is perhaps inadvisable to stress the significance of this finding, since the possibility of considerable variation from plant to plant, or sample to sample, in

a crop such as kale must be kept in mind. The data suggest, however, that during the winter a gradual transformation of invert sugar into sucrose may be taking place in the marrow, and this feature of the composition of marrow stem kale will be examined in greater detail in a future investigation.

*Fractionation of marrow stem kale into leaf, rind and marrow.* On October 15 the leaves from a number of representative plants from the singled-out marrow stem kale were severed at the junction with the main stem. The stems were further fractionated into rind and marrow, and the wet and dry weights of leaf, rind and marrow were determined. The results are shown in Table II. A complete analysis of the three fractions was also made, the results in this case being given later in Table VI.

Table II. *Fractionation of singled-out marrow stem kale into leaf, rind and marrow (October 15, 1934)*

	% dry matter	Dry matter in fractions as % of dry matter of whole plant	Proportions
Leaf	12.20	55.0	5.4
{ Rind	16.54	34.8	3.4
{ Marrow	8.57	10.2	1.0
Stem	13.66	45.0	4.4

It will be seen from Table II that 55 per cent. of the total dry matter of the singled-out marrow stem kale was present in the leaf and 45 per cent. in the stem. The very moist and succulent marrow contained 10.2 per cent. of the dry matter in the whole plant, whilst the hard rind, which was the driest of the three fractions, accounted for as much as 34.8 per cent. On the basis of the sugar percentages in the first column in Table I, it may be shown that 4.18 per cent. of the total dry matter of the crop consisted of reducing sugar and 1.11 per cent. of sucrose, making a total sugar content of 5.29 per cent.

The view was held that the sugar-rich character of the marrow in marrow stem kale was traceable possibly to the influence of the kohlrabi parent, and that the marrow from the stems of thousand head kale might therefore not exhibit, in such marked degree at any rate, this characteristic of high sugar content. Further investigation, however, proved this view to be incorrect, as will be clear from the data in Tables III and IV. It may be pointed out that the determinations were carried out on plants of thousand head kale with stems of  $\frac{3}{4}$ –1 $\frac{1}{2}$  in. thickness, the occasional thin-stemmed plants ( $\frac{1}{4}$  in. thickness) being excluded from

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the samples. By this means the results were made more comparable with the corresponding figures for singled-out marrow stem kale in Tables I and II than otherwise would have been the case.

Table III. *Fractionation of thousand head kale into leaf, rind and marrow (November 25, 1934)*

	% dry matter	Dry matter in fractions as % of dry matter of whole plant	Proportions
Leaf	14.00	58.7	5.4
{ Rind	26.33	30.5	2.8
{ Marrow	15.56	10.8	1.0
Stem	22.29	41.3	3.8

Table IV. *Dry-matter and sugar content of marrow from thousand head kale*

Date of sampling	Nov. 18, 1934	Nov. 20, 1934	Feb. 14, 1935
Dry matter (%)	13.30	13.11	14.26
On basis of dry matter:			
Reducing sugar (%)	25.20	29.90	29.24
Sucrose (%)	20.90	17.67	14.67
Total sugar (%)	46.10	47.57	43.91

A comparison of the data in Tables II and III suggests that the thousand head kale was slightly leafier than the singled-out marrow stem kale, but the significance of this comparison is much reduced by the obvious difficulty of securing comparable samples from such non-uniform crops. Despite the swollen character of the stems of the marrow stem kale, the marrow from the thousand head kale formed quite as high a proportion of the dry matter of the whole plant as was the case with the marrow stem kale. Indeed, the main distinction between the two kales lay in the distinctly higher percentages of dry matter in the marrow and rind, and therefore in the whole stem, of the thousand head kale, and although this difference may be due in part to the fact that the two kales were sampled for the determinations at very different dates of the winter, it will nevertheless be noted that in the case of the marrow, all the determinations consistently showed a higher percentage of dry matter in the marrow of the thousand head variety (see Tables I, II, III and IV). It has, moreover, been shown already that the whole plants of thousand head kale were characterised by a higher dry-matter content than those from the marrow stem kale.

In respect of sugar content, it will be seen from Tables I and IV that the marrow from the thousand head kale was almost as rich as that

from the marrow stem kale, nearly half of the dry matter of the marrow being composed of a mixture of sucrose and invert sugar. The preponderance of reducing sugar over sucrose was not nearly so pronounced as in the marrow from the October sample of marrow stem kale, but this may have been due to sampling the thousand head kale during November, when the crop had been subjected to a number of night posts. The effect of low temperature on the distribution of the marrow sugar between sucrose and reducing sugar will be examined further in future investigations, but it is scarcely possible that the observed differences represent a real distinction between the two varieties of kale, particularly in view of the finding that the distribution in the case of the marrow stem kale had undergone such pronounced modification by the date of the final determinations in January (see Table I).

From the foregoing findings it appears justifiable to infer that the characteristic of high sugar content in the marrow of marrow stem kale is inherited from the thousand head kale rather than from the kohl-rabi parent. Unfortunately the writers have been unable to discover any reference to recent determinations of the sugar content of kohl-rabi, so that it is not possible, in the absence of such information, to speculate on the influence of the kohl-rabi parent in this connection. Ingle<sup>(11)</sup>, however, quotes an old result of König to the effect that the kohl-rabi "bulb", on the basis of dry matter, contains only about 3 per cent. of sugar. If this figure may be taken as reliable, it appears correct to attribute the high sugar content of the marrow in marrow stem kale to the influence of the thousand head kale parentage. But before deciding this point finally, further work on the composition of kohl-rabi is desirable.

*Composition of the dry matter.* The data for the composition, both organic and inorganic, of the composite samples of marrow stem kale (both unthinned and singled out) and thousand head kale are given in Table V. The results are expressed on the basis of dry matter, and the periods of the season are also shown over which the daily cuts of kale were taken for the purposes of the digestion trials and the making of the composite samples.

In addition to the analytical data for whole marrow stem kale, the composition of the separate fractions of leaf, marrow and rind from singled-out marrow stem kale was also investigated. These results, which are recorded in Table VI, are of interest in illustrating the distribution of the constituents in the various parts of the plant, but must not be taken as being strictly comparable with the data for the whole plant

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Table V. *Composition, on basis of dry matter, of composite samples of marrow stem kale and thousand head kale*

	Marrow stem kale (unthinned)		Marrow stem kale (singled-out)		Thousand head kale	
Date of cutting ...	Sept 22- Oct. 1	Jan. 9-18	Oct. 22-31	Jan. 29- Feb. 9	Nov. 12-21	Feb. 19-28
Digestion period ...	1	5	2	6	3	7
	%	%	%	%	%	%
Crude protein	16.19	14.64	13.20	16.01	11.29	15.81
Ether extract	3.98	2.81	2.49	2.32	2.22	2.56
N-free extractives	46.35	52.72	52.24	51.15	58.07	49.07
Crude fibre	18.32	18.34	18.55	17.88	18.48	21.29
Ash	15.16	11.49	13.52	12.64	9.94	11.27
SiO <sub>2</sub> -free ash	14.86	11.39	13.43	12.45	9.58	11.24
Lime (CaO)	3.72	2.45	3.23	2.52	2.51	2.39
Phosphoric acid (P <sub>2</sub> O <sub>5</sub> )	0.73	0.90	0.74	0.87	0.64	0.99
Chlorine (Cl)	1.45	1.29	1.79	1.38	0.90	1.11
Potash (K <sub>2</sub> O)	4.03	3.89	3.84	4.37	2.67	3.88
Soda (Na <sub>2</sub> O)	0.51	0.26	0.37	0.21	0.21	0.14
Magnesia (MgO)	0.52	0.39	0.43	0.66	0.48	0.44
Total sulphur	1.35	1.10	1.22	0.97	0.93	1.04
Inorganic sulphur	0.79	0.51	0.74	0.52	0.49	0.45
Organic sulphur	0.56	0.59	0.48	0.45	0.44	0.59
True protein	12.25	10.23	9.68	10.68	9.22	10.60
"Amides"	3.94	4.41	3.52	5.33	2.07	5.21
Iron oxide (Fe <sub>2</sub> O <sub>3</sub> )*	0.0084	—	0.0062	—	0.0093	—

\* The iron determinations were made on separate samples of kale, precautions being taken to avoid all possible contamination with iron during sampling and preparing the sample for analysis.

Table VI. *Separate composition, on the basis of dry matter, of leaf, marrow and rind from singled-out marrow stem kale*

	Leaf %	Marrow %	Rind
Crude protein	13.87	12.03	7.72
Ether extract	3.33	1.34	0.81
N-free extractives	55.49	58.38	55.34
Crude fibre	12.45	13.06	29.08
Ash	14.86	15.19	7.05
SiO <sub>2</sub> -free ash	14.67	15.19	7.05
Lime	5.00	1.34	1.25
Phosphoric acid	0.60	1.06	0.61
Chlorine	1.94	2.02	0.74
Potash	2.45	5.48	2.47
Soda	0.24	2.00	0.43
Magnesia	0.37	0.53	0.40
Total sulphur	1.48	1.09	0.70
Inorganic sulphur	0.93	0.57	0.28
Organic sulphur	0.55	0.52	0.42
True protein	12.84	8.05	6.03
"Amides"	1.03	3.98	1.69

(singled-out) as shown in Table V. The fractional analyses were carried out on material from a separate sampling of the singled-out crop, and owing to non-uniform growth and consequent great variation in composition from plant to plant, the taking of representative and comparable samples from a crop of marrow stem kale is a matter of extreme difficulty. This question will be referred to again in the discussion of the analytical results.

#### *Comments on Tables V and VI*

*Protein content.* The September figures for the marrow stem kale (unthinned) were obtained when the crop was at its best. It was remarkably fresh and green, with very few brown leaves, and as yet untouched by frost. The general height of the crop was about 30 in., the highest plants, however, reaching 42 in. The plants were in all stages of development, from poorly grown plants with stems no more than  $\frac{1}{4}$  in. thick to well-grown plants with stems of 2 in. thickness. Most of the stems, however, were rather more than 1 in. thick.

An outstanding feature of the marrow stem kale at this stage was its richness in crude protein, the latter amounting, on the dry-matter basis, to 16.19 per cent. and being made up of 12.25 per cent. of true protein and 3.94 per cent. of "amides". When the second composite sample of unthinned marrow stem kale was made in the following January, the crude protein figure had fallen to 14.64 per cent. A corresponding decline was shown in the percentage of true protein, whereas the figure for "amides" displayed a slight increase.

This change in protein content is considered significant despite the recognised non-uniform growth of the crop over even small areas. It arose from the loss of leaf that was taking place during the winter, numerous leaves, especially on the smaller plants, turning wilted and brown and subsequently falling off. An examination of the data in Table VI shows the leaves to be richer in both crude and true protein, but poorer in "amides", than the stems, so that the observed changes in protein content are consistent with the explanation that these were caused by loss of leaf.

It will be noted that the singled-out marrow stem kale cut during October had a lower protein content (13.20 per cent. of the dry matter) than the unthinned crop. The cause of this difference lay in the lower content of true protein rather than in any difference in the "amide" content. It is a distinction that would be anticipated on the basis of the much greater development of the stems in the singled-out kale and



the absence of immature, thin-stemmed plants such as were common in the unthinned crop.

The increased crude-protein content (16.01 per cent. of the dry matter) observed later in the winter, when the crop was cut daily in January and February, is difficult to account for. This behaviour was the reverse of that noted in the case of the unthinned crop. It cannot be explained on the basis of increased leafiness, since on an average at this stage the leaf, on the wet basis, formed only 45 per cent. of the total crop, compared with 58 per cent. in the previous October. It will be seen from Table V that the "amides" contributed more largely to this increase in crude protein than did the true protein, and it is possible, therefore, that the change was brought about by an uptake of nitrogen, particularly during the abnormally mild, wet days of December, and that this nitrogen had mainly been stored in the marrow as "amides", the conditions not being such as to favour its transformation into the protein of new leaf growth. On the other hand, however, the possibility must be kept in mind that the difference was due to lack of strict comparability in the growths on the two areas from which the kale was cut. The non-uniform growth, even on contiguous small areas, is illustrated by the following series of successive daily determinations of the percentage of leaf (wet basis) in the samples of singled-out marrow stem kale cut during January and February: 51, 41, 49, 43, 39, 38, 44, 37, 34, 53, 43, 51, 49, 52, 47, 52, 43, 46 per cent. It should be emphasised that these data do not merely represent a plant to plant variation, but that each figure was obtained by the fractionation of a sample made up from a large number of plants.

The distribution of the nitrogenous components in the different parts of the singled-out marrow stem kale is shown in Table VI. The leaf was richest in crude protein (13.87 per cent. of the dry matter), the percentage of non-protein nitrogenous matter being quite small (1.03 per cent.). The rind contained the smallest percentage of crude protein (7.72 per cent.), while the marrow (12.03 per cent.) was only slightly poorer than the leaf. The marrow, however, was much richer in "amides" than either the leaf or rind, no less than 3.98 per cent. of its dry matter being composed of this simple form of nitrogen. It has already been shown that the juice expressed from the marrow evolved nitrogen freely when tested with alkaline hypobromite.

That the crude-protein content of kale is not determined wholly by the leafiness of the crop is again brought out by the results for the two composite samples of thousand head kale. In November the percentage

of leaf in the crop (wet basis) was 64 per cent. Despite this high value, however, the percentage of crude protein in the dry matter of the kale was only 11.29 per cent., a low value when compared with that for the singled-out marrow stem kale, in which the swollen stems formed a higher proportion of the whole plant. When the thousand head kale was again investigated in the following February, the percentage of leaf (wet basis) had fallen to 60 per cent., but the crude protein, on the dry-matter basis, had risen to 15.81 per cent. The rise, however, was largely accounted for by a very distinct increase in the percentage of "amides" (2.07-5.21 per cent.), and may therefore have been occasioned by an uptake of nitrogen from the soil during the mild, wet December, such nitrogen having been stored for the most part in the marrow as "amides" (*e.g.* asparagine), the conditions not having been such as to lead to its conversion into true protein. Under conditions favourable to renewed growth, such stored-up "amides" would be utilised for the elaboration of protein in the new plant tissues.

*Content of ether extract, crude fibre and N-free extractives.* As with most green crops, the amount of ether extract in both kinds of kale was quite small, the percentage varying, on the basis of dry matter, between 2 and 3 per cent. in five of the six composite samples. Only in the case of the unthinned marrow stem kale at the earliest stage of the work in September, when the crop was at its greenest and healthiest, did the amount rise beyond 3 per cent. In this sample, the percentage rose to about 4 per cent. of the dry matter.

Reference to Table VI shows that in the case of the singled-out marrow stem kale, the leaves (3.33 per cent. on the dry-matter basis) were distinctly richer in ether extract than the other parts of the plant. Next in richness came the marrow and finally the rind, with but 0.81 per cent.

There was no significant difference in fibre content between the unthinned and singled-out marrow stem kale. Neither was there any indication of any increase in fibre content as the winter advanced, the percentages, on the basis of dry matter, for all the marrow stem kale samples varying within the narrow limits of 17.9-18.6 per cent. In the case of the thousand head kale, the first composite sample (November) contained an almost equal percentage of crude fibre (18.5 per cent.), but this had increased to 21.3 per cent. when the second composite sample was made in the following February.

It is interesting to note that the most fibrous part of the marrow stem kale was the rind, the dry matter of which contained as much as

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29.1 per cent. of crude fibre. The marrow, somewhat against expectations, proved to be slightly more fibrous than the leaves (13.06 per cent. of the dry matter as against 12.45 per cent.), but this may have in part been due to the unavoidable admixture of a small amount of the woody xylem with the marrow during the fractionation of the plants.

There was little of importance to distinguish the various kale composite samples in respect of N-free extractives, the most interesting observation having already been referred to, namely, that almost the whole of the N-free extractives in the marrow of marrow stem kale can be accounted for in terms of sucrose and reducing sugar.

*Mineral content.* One of the most important observations in this investigation is the discovery of the very high ash content (14.9 per cent. of silica-free ash on the dry-matter basis) of the September sample of unthinned marrow stem kale. This was distinguished by its richness in lime (3.72 per cent. of the dry matter), chlorine (1.45 per cent.), potash (4.03 per cent.) and total sulphur (1.35 per cent.). The amount of phosphoric acid, on the other hand, was but moderate (0.73 per cent.). The significance of these data will be better appreciated by comparing them with similar data for pasture grass (12, 13), lucerne (14) and swedes (2).

Table VII. *Mineral composition of marrow stem kale, pasture grass, lucerne and swedes (dry-matter basis)*

	Unthinned marrow stem kale (September composite) %	Pasture grass (mown at monthly intervals; May grass) %	Lucerne (early flower) %	Swedes %
SiO <sub>2</sub> -free ash	14.86	8.23	10.44	6.09
Lime	3.72	1.32	4.76	0.70
Phosphoric acid	0.73	1.11	0.66	0.70
Potash	4.03	3.75	2.34*	2.61
Soda	0.51	0.20	0.40	
Magnesia	0.52		0.42	
Total sulphur	1.35	0.63	0.59	
Inorganic sulphur	0.79	0.31	0.11	
Organic sulphur	0.56	0.32	0.48	
Chlorine	1.45	1.01	0.44	0.35
Iron oxide	0.0084		0.012	

\* Variable within fairly wide limits.

Note. The empty spaces in Table VII indicate absence of information.

The data in Table VII emphasise the mineral-rich character of the September composite of unthinned marrow stem kale, the latter containing an appreciably higher percentage of SiO<sub>2</sub>-free ash than either grass or lucerne. In respect of lime, for which lucerne enjoys a special reputation, the kale gave a figure (3.7 per cent. of the dry matter) of

the same order as that for lucerne and very much higher than that for pasture herbage.

It has been found in recent Danish trials<sup>(7)</sup> that as much as 120 lb. per head per day of marrow stem kale may be fed to dairy cows without causing any undesirable flavour in the milk. Such an allowance would supply, on the basis of the data under consideration, about  $9\frac{1}{2}$  oz. of lime, *i.e.* an amount, on the usual assumption of 50 per cent. absorption, sufficient for maintenance and the production of about 16 gallons of milk. These figures demonstrate the value of kale in milk rations and its useful function of supplying an abundance of lime to balance the phosphoric acid of the concentrate mixture, the latter as a rule tending to be rich in phosphoric acid and deficient in lime. It is clear also that when sheep are folded on kale and given, in addition, a small allowance of hay and concentrates, no anxiety need be felt concerning the lime supply.

The data in Table VI reveal the fact that the leaf of the kale is the main seat of the lime, the dry matter of the leaf containing no less than 5.0 per cent., compared with 1.34 and 1.25 per cent. respectively in the marrow and rind. This finding accounts for the somewhat lower lime content (3.23 per cent. of the dry matter) of the October sample of singled-out kale, with its greater development of stem in relation to leaf. The running off of lime content in both the unthinned and singled-out kale as the winter went by is also consistent with the wilting and falling off of a proportion of the leaves. Even at its phase of lowest lime content, however, an allowance of 120 lb. of the marrow stem kale would have supplied sufficient lime for maintenance of a dairy cow and the production of about 9 gallons of milk.

Marrow stem kale is not particularly distinguished from grass and lucerne in respect of its content of phosphoric acid, the data for this constituent ranging from 0.73 to 0.90 per cent. of the dry matter. As with lucerne, the phosphoric acid is low in relation to the lime content. Other outstanding features of the mineral composition of marrow stem kale are the high percentages of potash, chlorine and sulphur, both organic and inorganic, the presence of the last-named constituent conferring an advantage on kale as a sheep feed from the standpoint of wool growth.

Lucerne is usually regarded as a satisfactory source of iron, containing about 12 mg.  $\text{Fe}_2\text{O}_3$  per 100 gm. of dry matter. The corresponding data for the unthinned and singled-out kale were 8.4 and 6.2 mg. per 100 gm. of dry substance.

The superiority of marrow stem kale over swedes in minerals, particularly in respect of lime and chlorine, is noteworthy (see Table VII).

The data in Table VI reveal several interesting facts concerning the distribution of the minerals in marrow stem kale. The leaves are richest in lime and inorganic sulphur, while the marrow showed the highest percentages of phosphoric acid, potash, soda and magnesia. The percentages of chlorine and organic sulphur in the leaf and marrow were of the same order. The rind, although in general poorer in minerals than both leaf and marrow, is nevertheless to be looked on as making quite a significant contribution to the nutritive value of the whole plant from the standpoint of mineral supply.

Reference to Table V shows that thousand head kale, although slightly less rich in  $\text{SiO}_2$ -free ash than the marrow stem variety, displays the same general features of mineral composition. Again are to be noted the high percentages of lime, potash and sulphur, with satisfactory percentages of phosphoric acid, chlorine, magnesia and iron. From the standpoint of mineral supply, there can be little doubt that the two varieties would prove almost equally satisfactory in practice.

#### DIGESTIBILITY AND NUTRITIVE VALUE OF MARROW STEM KALE AND THOUSAND HEAD KALE

Six digestion trials of the kales were carried out in the order already shown in an earlier section dealing with the scope of the present investigation, the object being not only to compare the digestibilities of the two varieties of kale, but also to ascertain the effect of winter conditions on composition, digestibility and nutritive value.

Two pure-bred Suffolk wethers, aged about 18 months at the commencement of the trials, were used for the purpose of the measurements. In the first period (September 1934), the digestibility of the unthinned marrow stem kale was determined. The ration consisted of 9000 gm. of kale fed in three equal parts during the day. With the object of simplifying sampling and also securing, as far as possible, non-selective feeding, the kale before feeding was put through a chaffing machine so that the stems were cut into lengths varying from 1 to 2 in. No difficulty was experienced in securing complete consumption of the kale rations throughout this period. It should be noted that in all the digestion periods, any brown leaves on the plants were included in the samples fed to the sheep, since such leaves would be consumed by sheep folded on kale.

The second period of investigation (October 1934) was devoted to

the determination of the digestibility of the singled-out marrow stem kale. At the beginning of this period it became apparent that the sheep were growing tired of a ration composed wholly of kale. They began to refuse the food and to lose their appearance of general well-being. The difficulty was readily overcome by including an allowance of good meadow hay in the ration and cutting down the allowance of kale. A rock salt lick was also placed in the feeding troughs. Under this treatment the animals quickly improved in appetite and condition, and no difficulty was experienced in securing full consumption of a ration composed of 300 gm. of chaffed meadow hay and 6000 gm. of kale. It was discovered that the kale was most relished when the leaves were stripped from the plants and fed whole, and the thick stems were cut into lengths of about 1 in. This plan of feeding was followed successfully in all the remaining kale digestion periods.

The digestibility of the thousand head kale was next determined in a third period (November 1934), this being succeeded by a feeding trial in which the digestion coefficients of the meadow hay (daily ration = 1300 gm. of chaffed hay) were ascertained. With the advent of the New Year, the digestion measurements on the three kale crops were repeated, the unthinned marrow stem kale being tested in January 1935, the singled-out marrow stem kale at the end of January and beginning of February 1935 and the thousand head kale in late February 1935.

Table VIII. *Digestibility and nutritive value of marrow stem kale and thousand head kale*

Digestion period	Marrow stem kale (unthinned)		Marrow stem kale (singled-out)		Thousand head kale	
	1	5	2	6	3	7
Dates corresponding with composite samples of kale	1934 Sept. 22- Oct. 1	1935 Jan. 9-18	1934 Oct. 22-31	1935 Jan. 29- Feb. 9	1934 Nov. 12-21	1935 Feb. 19-28
Digestion coefficients of:	%	%	%	%	%	%
Crude protein	81.6	76.3	78.1	78.8	74.3	79.4
Ether extract	59.8	64.9	51.3	47.9	35.4	50.0
N-free extractives	88.0	88.3	89.5	88.0	90.7	86.9
Crude fibre	66.6	61.9	62.0	54.2	57.9	59.3
Total organic matter	80.8	80.1	80.8	78.3	80.5	77.9
On basis of dry matter:						
Digestible crude protein	13.21	11.17	10.31	12.62	8.39	12.55
Digestible ether extract	2.38	1.82	1.28	1.11	0.79	1.28
Digestible N-free extractives	40.79	46.55	46.75	45.01	52.67	42.64
Digestible fibre	12.20	11.35	11.50	9.69	10.70	12.62
Total digestible organic matter	68.58	70.89	69.84	68.43	72.55	69.09
Starch equivalent*	64.65	66.56	65.00	63.50	67.41	63.33
Protein equivalent	11.24	8.97	8.55	9.95	7.35	9.94
Nutritive ratio	4.4	5.5	5.9	4.5	7.8	4.7

\* Not allowing lower starch value for the sugar in the kale.

The complete details of the several digestion trials are given in the tables forming the Appendix to this paper. The data respecting the digestibility and nutritive value of the kales are summarised in Table VIII. For the analytical data of the composite samples of kale, the reader is referred to Table V. The meadow hay fed in conjunction with the kale in all the periods save the first contained, on the basis of dry matter, 13·25 per cent. of crude protein, 1·31 per cent. of ether extract, 47·59 per cent. of N-free extractives, 26·99 per cent. of crude fibre and 10·86 per cent. of ash.

*Comments on Table VIII*

The data for the unthinned marrow stem kale (digestion period 1) show that this green fodder is very digestible. The high digestion coefficients for the crude protein (81·6 per cent.), N-free extractives (88·0 per cent.) and total organic matter (80·8 per cent.) may be cited in support of this statement, such values being characteristic of concentrated foods rather than of coarse fodders or green fodders. Further emphasis to this finding is given by the data in Table IX, in which the data for marrow stem kale are compared with corresponding figures for green oats and vetches(15), lucerne in flower(16), medium meadow hay(17), sugar-beet tops(18) and pasture grass cut at monthly intervals(19).

Table IX. *Comparison of digestibility of unthinned marrow stem kale (late September) with that of other green fodders*

	Marrow stem kale	Green oats and vetches	Lucerne in flower	Sugar- beet tops	Monthly cut pasture grass	Medium meadow hay
Digestion coefficients of:	%	%	%	%	%	%
Crude protein	81·6	63·1	75·9	70·2	76·0	50·0
Ether extract	59·8	51·9	14·0	62·8	55·9	30·0
N-free extractives	88·0	76·5	66·8	82·6	82·7	53·1
Crude fibre	66·6	47·6	44·7	71·1	80·0	52·4
Total organic matter	80·8	65·5	60·2	78·5	80·7	51·9

The data in Table IX show that marrow stem kale is superior in digestibility to green oats and vetches, flowering lucerne and medium meadow hay, and compares favourably in this respect with sugar-beet tops and pasture grass cut at monthly intervals. This very satisfactory result is in complete harmony with the results of continental trials, in which the digestion coefficient of the organic matter in marrow stem kale has been found to range from 75 to 87 per cent. (mean of extremes = 81 per cent.)(7).

A further notable feature of the data in Table VIII is the proof they afford of the striking similarity in digestibility and feeding value between the unthinned and singled-out marrow stem kale, and between these crops and the thousand head kale. It is also a finding of importance that with the progress of winter, none of these crops suffered any serious deterioration in digestibility and feeding value, as measured by the digestion coefficients and by their content of digestible organic matter and starch equivalent. In the case of the singled-out marrow stem kale this finding was of particular interest, since severe snowstorms and frosty conditions had been prevalent just prior to the beginning of the digestion measurements on this crop at the end of January 1935. The most definite changes were in the figures for the percentages of digestible crude protein, but these were related to those variations in crude-protein content that have been considered earlier in this paper rather than to any marked changes in the digestion coefficients of this constituent.

In Table X, the data (mean of the two separate determinations) for the kale crops are compared with corresponding figures for other succulent crops that might also be available on the farm during winter for feeding to live stock, namely, swedes(2), mangolds(2), oat and vetch silage(20), maize silage(21) and sugar-beet tops(18).

Table X. *Comparison of feeding value of marrow stem kale and thousand head kale with that of other succulent foods available for winter feeding*

	Marrow stem kale (un- thinned)	Marrow stem kale (singled- out)	Thou- sand head kale	Swedes	Man- golds	Oat and vetch silage (acid brown)	Maize silage (Jaune Gros)	Sugar beet tops
On basis of dry matter:	%	%	%	%	%	%	%	%
Starch equiv.	65.6	64.3	65.4	63.5	51.7	33.4	57.9	52.8
Protein equiv.	10.1	9.3	8.7	6.1	3.3	8.2	5.0	7.1
Dry matter in fresh crop	13.3- 14.2	13.5- 14.2	15.7- 16.0	10-14	10-13	34.6	21.0	16.2

The data in Table X show that for purposes of winter keep, both varieties of kale have much to recommend them. The nutritive value of the dry substance in these crops, as expressed in terms of starch equivalent, is very distinctly superior to that of an equal weight of the dry substance in mangolds, sugar-beet tops, maize silage and "acid brown" oat and vetch silage.

The close agreement between the starch equivalent, per 100 lb. of dry matter, of the kales and of swedes is very striking. It may be inferred from Table X that 1 ton of the dry matter in either marrow stem or thousand head kale is very nearly equal in starch equivalent to



1 ton of the dry matter in swedes. Because of this equality on the dry-matter basis, the relative feeding values of green kale and swedes must be determined by the relative dry-matter contents of the crops. In this respect, the kale crops, particularly the thousand head variety, have the advantage, the percentage of dry matter in the kales usually being higher than in swedes. Where a swede crop has the high dry-matter content of about 14 per cent., 1 ton of such swedes may be taken as supplying as much starch equivalent as 1 ton of marrow stem kale; but in the case of swedes of lower dry-matter content, the advantage lies with the kale. For example, on the basis of the data in Table X, it may be concluded that 1 ton of green marrow stem kale (containing 13.8 per cent. of dry matter) supplies as much starch equivalent as about  $1\frac{1}{3}$  tons of swedes (containing 11.5 per cent. of dry matter), while 1 ton of thousand head kale (containing 15.8 per cent. of dry matter) is equal, from the starch equivalent standpoint, to about  $1\frac{1}{3}$  tons of such swedes.

Considerations of soil and climate must naturally be the factors on which the farmer bases his choice between the growing of kale and of swedes for the purposes of winter keep. Where the land is capable of producing a good crop of kale, however, this crop offers attractive possibilities. As a source of digestible protein, both varieties of kale are distinctly superior to swedes. Indeed, the kales are to be regarded as a valuable source of this constituent. A crop of 20 tons per acre of marrow stem kale would yield, on the basis of the data in Table X for the unthinned crop, 6180 lb. of dry substance, including 4054 lb. of starch equivalent and 624 lb. of protein equivalent (*i.e.* 753 lb. of digestible crude protein including 258 lb. of "amides"). The satisfactory magnitude of this yield, from the standpoints of both starch equivalent and digestible protein, is seen by comparing the figures with the yield of nutrient matter obtained from a Cambridge pasture, in a summer of favourable rainfall distribution, by a system of monthly cuts under conditions of intensive fertilising. Over the whole season (mid-April to beginning of October) the pasture yielded, per acre, 5762 lb. of dry matter, including 3860 lb. of starch equivalent and 812 lb. of digestible crude protein<sup>(19)</sup>. It is only fair to add, however, that the pasturage, which was situated on a light, sandy soil, was only of moderate quality, and that the yields in this case by no means represent the full productive capacity that has been realised on good permanent pasturage on a heavy clay soil<sup>(12)</sup>.

It has already been shown earlier in this paper that both varieties of kale are notably superior to swedes as a source of minerals, particularly

in respect of lime and chlorine. The use of kale as a winter food has a particular advantage in that it ensures a supply of carotene for maintaining the desired colour in milk and butter at a time when dairy cows frequently have no access to pasturage. Kale is also valuable as an all-round source of vitamins, but it should also be noted in this respect that swedes are also a useful food for inclusion in the rations of dairy cows in winter on account of their high content of vitamin C.

It may be objected that the too liberal use of kale in the rations of dairy cows is liable to produce a taint in the milk. The same objection, however, applies to swedes. The risk in both cases may be minimised by feeding either kale or swedes immediately after, and never just before, the milking operation. It is interesting to note that in Edin's experiments at continental stations, as much as 120 lb. of marrow stem kale has been fed per cow daily without producing any undesirable flavour in the milk<sup>(7)</sup>. Feeding experiments in Germany<sup>(10)</sup> have established the value of marrow stem kale for dairy cows up to a limit of 88 lb. per head per day, but the conclusion was drawn that special care is needed in feeding it if tainting of the milk is to be avoided. Being richer in protein than mangolds or swedes, less protein need be supplied in the concentrates when using marrow stem kale than when roots are used. On the basis of the present data for unthinned marrow stem kale (see Table X), it will be seen that an allowance of 90 lb. of marrow stem kale would supply 12.4 lb. of dry matter, including 8.1 lb. of starch equivalent and 1.25 lb. of protein equivalent; *i.e.* sufficient starch equivalent, in the case of a Shorthorn cow of 10 cwt. live weight, for maintenance and the production of about  $\frac{2}{3}$  gallon of milk, and sufficient protein equivalent for maintenance and rather less than 1 gallon of milk.

#### SUMMARY

The paper records the results of an investigation into the composition, digestibility and nutritive value of marrow stem kale (both unthinned and singled-out) and thousand head kale. A number of the main findings are recorded below.

The average dry-matter content of unthinned marrow stem kale during September and October was 13.3 per cent. During the following January it had reached a slightly higher level, namely, 14.2 per cent. Singling out of the marrow stem kale appeared to exercise little effect on the dry-matter content of the crop. Thousand head kale, with an average dry-matter content of 15.8 per cent., is significantly richer in dry matter than the marrow stem kale, although this advantage was

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offset by the lower yield, in terms of green matter, given per acre by the thousand head kale.

It was found that 55 per cent. of the total dry matter of the singled-out marrow stem kale was contained in the leaves of the plant, 34·8 per cent. in the rind and 10·2 per cent. in the marrow. The marrow contained less than 10 per cent. of dry matter, of which, during October, rather more than half was composed of a mixture of invert sugar and sucrose in the proportion of about 4 to 1. Nearly the whole of the N-free extractives of the marrow was in the form of sugar. The marrow from thin as well as thick stems of marrow stem kale displayed this sugar-rich character. Determinations made in the following January revealed the same high content of sugar in the marrow, but at this date the sucrose was present in slight excess of the reducing sugar.

The marrow in thousand head kale had a distinctly higher percentage of dry matter than that in the stems of thousand head kale and was almost as rich in sugar, nearly half of the dry matter being composed of sucrose and invert sugar.

An outstanding feature of the unthinned marrow stem kale in September was its richness in protein, this amounting, on the basis of dry matter, to 16·19 per cent. and being made up of 12·25 per cent. of true protein and 3·94 per cent. of "amides". The protein content of the crop fell slightly as the winter advanced in consequence of loss of leaf. Evidence is brought forward, however, to show that changes in the crude-protein content of kale during winter may not wholly be governed by changes in leafiness, but that under favourable conditions there may be an uptake of nitrogen from the soil leading to a further storage of "amides" in the marrow. The singled-out marrow stem kale and thousand head kale were also found to be similarly rich in protein.

There was no significant difference in fibre content between the unthinned and singled-out marrow stem kale, the percentages, on the basis of dry matter, for all the samples varying within the narrow limits of 17·9–18·6 per cent. Neither was there any indication of an increase in fibre content as the winter went by. The fibre content of thousand head kale was found to be of the same order as for the marrow stem variety.

An important finding in respect of the composition of the kales is the discovery of their very high content of mineral matter, which is distinguished by its richness in lime, chlorine, potash and sulphur. Satisfactory amounts of iron and magnesia, together with an amount of phosphoric acid about equal to that in flowering lucerne, are also present. As a source of minerals, the kale crops are very much superior to swedes.

Complete analyses of the separate fractions of leaf, rind and marrow from singled-out marrow stem kale showed that the leaves are richest in lime and inorganic sulphur, while the marrow contained, on the dry-matter basis, the highest percentages of phosphoric acid, potash, soda and magnesia, the percentages of chlorine and organic sulphur being of the same order in leaf and marrow.

The data from the digestion trials revealed the fact that unthinned marrow stem kale is highly digestible. It is superior in this respect to green oats and vetches, flowering lucerne and medium meadow hay, and compares quite well with sugar-beet tops and pasture grass cut at monthly intervals. A striking similarity in digestibility and starch equivalent was observed between the unthinned and singled-out marrow stem kale, and between these crops and the thousand head kale. It was also found that with the progress of winter, none of these crops suffered any serious deterioration in digestibility and feeding value, as measured by the digestion coefficients and by their content of digestible organic matter and starch equivalent. It should be pointed out, however, that although several periods of severe frost were encountered during the course of the trials, the winter of the experiment was, on the whole, mild and open, with an abundance of rain.

The results of the trials revealed a close agreement between the starch equivalent, per 100 lb. of dry matter, of the kales and of swedes. One ton of the dry matter in either marrow stem or thousand head kale is very nearly equal in starch equivalent to 1 ton of the dry matter in swedes. When, therefore, a swede crop has the high dry-matter content of about 14 per cent., 1 ton of such swedes may be taken as supplying as much starch equivalent as 1 ton of marrow stem kale, but in the case of swedes of lower dry-matter content, the advantage lies with the kale. For example, 1 ton of marrow stem kale (containing 13.8 per cent. of dry matter) supplies as much starch equivalent as about  $1\frac{1}{2}$  tons of swedes (containing 11.5 per cent. of dry matter), while 1 ton of thousand head kale (containing 15.8 per cent. of dry matter) is equal, from the starch equivalent standpoint, to about  $1\frac{1}{3}$  tons of such swedes.

In conclusion, the writers are glad to acknowledge the expert assistance of Messrs V. Thurlbourn and C. Bendall, in whose hands the care of the experimental animals was placed throughout the trials.

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# APPENDIX

## Digestion tables

### Sheep XXVI

	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extrac-tives gm.	Crude fibre gm.	Ash gm.
(1) Marrow stem kale (unthinned), Sept. 22-Oct. 1, 1934:							
Daily ration of kale	1163.00	986.69	188.29	46.29	539.05	213.06	176.31
Voided	259.85	197.42	34.56	18.88	68.35	75.63	62.43
Digested	903.15	789.27	153.73	27.41	470.70	137.43	113.88
Digestion coefficients (%)	77.66	79.99	81.65	59.21	87.32	64.50	64.59
(2) Marrow stem kale (singled-out), Oct. 22-31, 1934:							
Daily ration of kale	849.60	734.74	112.15	21.16	443.83	157.60	114.86
Daily ration of meadow hay	258.80	230.69	34.29	3.39	123.16	69.85	28.11
Total daily ration	1108.40	965.43	146.44	24.55	566.99	227.45	142.97
Total voided	279.78	224.55	38.87	14.24	89.70	81.74	55.23
Total digested	828.62	740.88	107.57	10.31	477.29	145.71	87.74
Digested from hay	171.15	157.43	21.34	—	87.54	45.55	13.72
Digested from kale	657.47	583.45	86.23	10.31	389.75	97.16	74.02
Digestion coefficients of kale (%)	77.39	79.41	76.89	48.72	87.81	61.65	64.44

### (3) Thousand head kale, Nov. 12-21, 1934:

	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extrac-tives gm.	Crude fibre gm.	Ash gm.
Daily ration of kale	971.40	874.84	109.67	21.57	564.09	179.51	96.56
Daily ration of meadow hay	261.00	232.65	34.58	3.42	124.21	70.44	28.35
Total daily ration	1232.40	1107.49	144.25	24.99	688.30	249.95	124.91
Total voided	313.78	253.55	45.00	18.92	91.82	96.21	60.23
Total digested	918.62	853.94	99.25	6.47	596.48	151.74	64.68
Digested from hay	172.60	158.71	21.52	—	88.29	48.96	13.83
Digested from kale	746.02	695.17	77.73	6.47	508.19	102.78	50.85
Digestion coefficients of kale (%)	76.80	79.46	70.88	30.00	90.09	57.26	52.06

### (4) Meadow hay (basal food), Nov. 22-Dec. 11, 1934:

	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extrac-tives gm.	Crude fibre gm.	Ash gm.
Daily ration of meadow hay	1120.10	998.46	148.41	14.67	533.06	302.32	121.64
Voided	380.58	318.30	56.06	15.86	154.18	92.20	62.26
Digested	739.52	680.16	92.35	—	378.88	210.12	59.36
Digestion coefficients of hay (%)	66.02	68.12	62.23	—	71.08	69.50	48.80

### Sheep XXVII

	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extrac-tives gm.	Crude fibre gm.	Ash gm.
Daily ration of kale	1163.00	986.69	188.29	46.29	539.05	213.06	176.31
Voided	240.44	181.18	34.75	18.31	61.27	66.85	59.26
Digested	922.56	805.51	153.54	27.98	477.78	146.21	117.05
Digestion coefficients (%)	79.32	81.64	81.54	60.44	89.63	68.62	66.39
(2) Marrow stem kale (singled-out), Oct. 22-31, 1934:							
Daily ration of kale	849.60	734.74	112.15	21.16	443.83	157.60	114.86
Daily ration of meadow hay	258.80	230.69	34.29	3.39	123.16	69.85	28.11
Total daily ration	1108.40	965.43	146.44	24.55	566.99	227.45	142.97
Total voided	262.78	203.33	35.56	13.15	74.67	79.95	59.45
Total digested	845.62	762.10	110.88	11.40	492.32	147.50	83.52
Digested from hay	170.06	157.56	22.00	—	87.25	48.31	12.50
Digested from kale	675.56	604.54	88.88	11.40	405.07	99.19	71.02
Digestion coefficients of kale (%)	79.52	82.28	79.25	53.87	91.27	62.30	61.83
(3) Thousand head kale, Nov. 12-21, 1934:							
Daily ration of kale	971.40	874.84	109.67	21.57	564.09	179.51	96.56
Daily ration of meadow hay	261.00	232.65	34.58	3.42	124.21	70.44	28.35
Total daily ration	1232.40	1107.49	144.25	24.99	688.30	249.95	124.91
Total voided	292.27	234.61	36.88	16.19	85.53	96.21	57.46
Total digested	940.13	872.88	107.37	8.80	602.77	153.74	67.45
Digested from hay	171.48	158.88	22.17	—	87.99	48.72	12.60
Digested from kale	768.65	713.80	85.20	8.80	514.78	105.02	54.85
Digestion coefficients of kale (%)	79.13	81.59	77.69	40.80	91.26	58.50	56.80
(4) Meadow hay (basal food), Nov. 22-Dec. 11, 1934:							
Daily ration of meadow hay	1120.10	998.46	148.41	14.67	533.06	302.32	121.64
Voided	380.58	318.30	56.06	15.86	154.44	93.23	67.56
Digested	739.52	680.16	92.35	—	377.62	209.09	54.08
Digestion coefficients of hay (%)	66.02	68.12	64.16	—	70.84	69.16	44.46

# *Digestion tables cont.*

## Sheep XXVI

	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extrac-tives gm.	Crude fibre gm.	Ash gm.
(5) Marrow stem kale (unthinned), Jan. 9-16, 1935:							
Daily ration of kale	850.80	753.05	124.56	23.91	448.54	156.04	97.75
Daily ration of meadow hay	258.00	229.98	34.19	3.38	122.78	69.63	28.02
Total daily ration	1108.80	983.03	158.75	27.29	571.32	225.67	125.77
Total voided	275.79	223.36	41.56	11.99	88.30	81.51	52.43
Total digested	833.01	759.67	117.19	15.30	483.02	144.16	73.34
Digested from hay	170.61	156.94	21.28	—	87.27	48.39	13.67
Digested from kale	662.40	602.73	95.91	15.30	395.75	95.77	59.67
Digestion coefficients of kale (%)	77.86	80.04	77.00	63.99	88.23	61.38	61.04
(6) Marrow stem kale (singled-out), Jan. 29-Feb. 9, 1935:							
Daily ration of kale	841.80	735.40	134.77	19.53	430.58	150.52	106.40
Daily ration of meadow hay	263.00	234.44	34.85	3.45	125.16	70.98	28.56
Total daily ration	1104.80	969.84	169.62	22.98	555.74	221.50	134.96
Total voided	295.79	241.73	42.06	14.50	99.05	95.12	54.06
Total digested	809.01	728.11	127.56	8.48	456.69	126.38	80.90
Digested from hay	173.92	159.98	21.69	—	88.96	49.33	13.94
Digested from kale	635.09	568.13	105.87	8.48	376.73	77.05	66.96
Digestion coefficients of kale (%)	75.44	77.26	78.56	43.42	87.49	51.19	62.93

## (7) Thousand head kale, Feb. 19-26, 1935:

	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extrac-tives gm.	Crude fibre gm.	Ash gm.
Daily ration of kale	970.80	861.39	153.48	24.85	476.38	206.68	109.41
Daily ration of meadow hay	264.00	233.33	34.98	3.45	125.65	71.25	28.67
Total daily ration	1234.80	1094.72	188.46	28.30	602.03	277.93	138.08
Total voided	336.54	278.45	46.37	11.91	101.85	112.32	58.09
Total digested	898.26	818.27	142.09	16.39	500.18	165.61	79.99
Digested from hay	174.59	160.00	21.77	—	89.31	49.52	13.99
Digested from kale	723.67	657.67	120.32	10.39	410.87	116.09	66.00
Digestion coefficients of kale (%)	74.54	76.35	78.39	41.81	86.25	56.17	60.32

## Sheep XXVII

	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extrac-tives gm.	Crude fibre gm.	Ash gm.
Daily ration of kale	850.80	753.05	124.56	23.91	448.54	156.04	97.75
Daily ration of meadow hay	258.00	229.98	34.19	3.38	122.78	69.63	28.02
Total daily ration	1108.80	983.03	158.75	27.29	571.32	225.67	125.77
Total voided	272.58	222.66	42.69	11.57	88.26	80.14	49.92
Total digested	836.22	760.37	116.06	15.72	483.06	145.53	75.85
Digested from hay	169.54	157.08	21.94	—	86.96	48.16	12.46
Digested from kale	666.68	603.29	94.12	15.72	396.06	97.37	63.39
Digestion coefficients of kale (%)	78.36	80.11	75.56	65.75	88.30	62.40	64.85
Daily ration of kale	841.80	735.40	134.77	19.53	430.58	150.52	106.40
Daily ration of meadow hay	263.00	234.44	34.85	3.45	125.16	70.98	28.56
Total daily ration	1104.80	969.84	169.62	22.98	555.74	221.50	134.96
Total voided	280.88	225.84	40.69	12.76	85.65	86.74	55.04
Total digested	823.92	744.00	128.93	10.22	470.09	134.76	79.92
Digested from hay	172.81	160.11	22.36	—	88.96	49.09	12.70
Digested from kale	651.11	583.89	106.57	10.22	381.43	85.67	67.22
Digestion coefficients of kale (%)	77.35	79.40	79.07	52.33	88.59	56.92	63.18
Daily ration of kale	970.80	861.39	153.48	24.85	476.38	206.68	109.41
Daily ration of meadow hay	264.00	233.33	34.98	3.45	125.65	71.25	28.67
Total daily ration	1234.80	1094.72	188.46	28.30	602.03	277.93	138.08
Total voided	312.62	251.81	42.63	13.84	95.75	98.59	61.01
Total digested	922.18	842.91	145.83	14.46	506.28	178.34	77.07
Digested from hay	173.48	160.73	22.44	—	89.01	49.28	12.75
Digested from kale	748.50	682.18	123.39	14.46	417.27	129.06	64.32
Digestion coefficients of kale (%)	77.10	79.43	80.39	58.19	87.59	62.44	58.80

# SELENIUM AS A CATALYST IN THE KJELDAHL METHOD AS APPLIED TO SOIL AND GRASS ANALYSIS

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## INTRODUCTION

THE search for some means of accelerating digestion in the Kjeldahl<sup>(1)</sup> method began in 1883 with Kjeldahl himself, who used phosphoric acid and fuming sulphuric acid for the purpose. His method also utilised the action of potassium permanganate to complete oxidation after the substance had been digested with strong sulphuric acid until a clear solution had been obtained. He regarded the addition of the permanganate as being indispensable and seems to have been influenced in this by Wanklyn<sup>(2)</sup> who, in 1868, had estimated nitrogen in organic compounds by distilling them with potassium permanganate and excess of alkali. He does not appear to have been aware of the work of Heintz and Raysky<sup>(3)</sup>, who, as early as 1875, had estimated urea by digestion with strong sulphuric acid.

The use of mercury and copper as catalysts was first recommended by Wilfarth<sup>(4)</sup> in 1885, while Arnold<sup>(5)</sup>, to whom the discovery is usually attributed, investigated the effect of adding benzoic acid, sugar, mercuric sulphate and copper, but did not publish his results until 1886. In 1889 Gunning<sup>(6)</sup> claimed that it was not a catalyst which was required, but some means of maintaining the strength of the acid, and he recommended for this purpose the use of potassium sulphate, which also raised the boiling point of the mixture. Although he considered that his modification made unnecessary the addition of a catalyst or potassium permanganate, the practice of adding catalysts soon arose, for in 1895 Dyer<sup>(7)</sup> published a paper in which he described a method employing both mercury and potassium sulphate to accelerate digestion. Nevertheless, the value of Gunning's modification was not immediately recognised, and for many years the original Kjeldahl method or the Kjeldahl-Gunning method was used according to the prejudice of the analyst.



However, Paul and Berry(8), in 1921, showed that the addition of potassium permanganate may cause losses of nitrogen, and in recent years the practice of adding it appears to have been dropped entirely.

A great many substances, including nickel, alumina, manganese, platinum and tungstic, molybdic, titanous and vanadic acids, have been tried as catalysts; but, with the possible exception of platinum, they have been found to have no advantage over copper and mercury and little progress was made until Lauro(9) published figures in 1930 which indicated that the clearing of the digested matter was accelerated considerably if selenium was substituted for mercury and copper. His paper aroused considerable interest; the modification was tried out in the analyses of a great many different substances, and some conflicting results were published. In 1934 Prince(10) was appointed by the Association of Official Agricultural Chemists (U.S.A.) as referee to investigate the matter. He compared the action of metallic mercury, selenium and mixtures of selenium and mercury and of selenium and copper sulphate, but made no comparison between selenium and copper sulphate. He paid no attention to the time taken to clear and based his conclusions upon the time taken to obtain maximum figures. He concluded that there was little to choose between the catalysts.

Previously it had been shown by Phelps and Daudt(11) in 1919 and by Paul and Berry(8) in 1921 that digestion was more rapid with mercury than with copper sulphate. As they did not accept clearing as a criterion that digestion was complete, their evidence appears to be fairly conclusive. If mercury is used, however, it is necessary before distilling to add sodium sulphide or sodium thiosulphate to liberate ammonia from the complex mercury-ammonium salts that are formed. It appears, therefore, that even if selenium is no more effective than mercury, there is a good reason why it should be preferred.

The disagreement as to the most suitable catalyst is not, however, as serious as the disagreement as to the time necessary to complete digestion. The early investigators carried on the digestion merely until they obtained a clear, more or less colourless, solution, and this is apparently still the standard practice in some laboratories. It is, however, becoming recognised that clearing is no criterion for deciding that the conversion of nitrogen to ammonium sulphate has been completed, and the practice of digesting for 1 hour after clearing is frequently adopted. With soils, however, digestion appears to be usually stopped as soon as the acid becomes "white" or "straw coloured". Müller(12), who first applied the method to soils, started this practice and it is recommended

by the Association of Official Agricultural Chemists<sup>(13)</sup>, although they recommend longer digestion periods for every other type of substance.

Further disagreement with regard to the digestion of soil has been occasioned by Bal's<sup>(14)</sup> discovery that the addition of water led to higher results with heavy soils. His work has been confirmed by Scrivinasan<sup>(15)</sup> and the addition of water appears to be a most necessary modification. The prior addition of water, however, interferes with the action of salicylic acid when this is added to fix nitrates, so that, under these circumstances, some other modification is necessary. As early as 1891 Ulsch<sup>(16)</sup> recommended the use of "reduced iron" for the reduction of nitrate in solution, but this causes bumping during distillation, and some means of combining Bal's modification with the salicylic acid method is obviously desirable.

Confronted by such a conflict of evidence and recommendations, the analyst has no choice but to make critical investigations of the recommended modifications under his own conditions of working. The present paper describes a series of these tests made over a period of two years. Comparisons between the catalytic action of copper sulphate and selenium when applied to Kjeldahl determinations on grass and on soil have been made. The length of the digestion period required to obtain a constant maximum value has been determined. Bal's modification has been used in all soil analyses, and a method of combining it with the salicylic acid method has been investigated. Comparisons between the salicylic acid method and the reduced iron method, using two different types of reduced iron, have also been made.

## EXPERIMENTAL

### *Description of apparatus and conditions of working*

It is thought advisable to include a brief description of the conditions of working so that they may be compared with those in other laboratories. Digestion was carried out in 500-ml. Kjeldahl flasks over "4-light" burners, using petrol gas supplied by a "Silverlite" generator. The full heat of these burners was applied directly to the digestion flasks, except for the nitrate modifications, when it was necessary to heat gently at first. The temperature reached during digestion was measured on several occasions by inserting a thermometer into the liquid in the flasks, and was found to be invariably in the neighbourhood of 345° C. "Refluxing" took place, after the first few minutes, from over half-way up the necks of the flasks.

## 242 *Selenium as a Catalyst in the Kjeldahl Method*

Previous investigators<sup>(9)</sup> have recommended the use of 0.2 gm. metallic selenium, and this was the weight used throughout the present investigation. A preliminary test showed that, whereas the clearing of the digested matter was not accelerated by using larger quantities, it took longer when smaller quantities were used. For example, samples digested with 0.1 and 0.05 gm. took clearing periods approximately 16 and 47 per cent. longer, respectively, than did those digested with 0.2 gm. The effect upon the total nitrogen figures, of adding greater and lesser weights of selenium, was not, however, investigated.

Blank determinations were made by digesting the reagents together with 1 gm. of sucrose for 5 hours.

### *Comparison of selenium and copper sulphate as catalysts in the Kjeldahl method when applied to grass*

All grass samples used in investigations reported in this paper were ground in a Christy and Norris Mill, using a sieve with holes 1/64 in. in diameter. Eleven batches, each of four 1-gm. portions of a sample of finely ground grass were weighed into Kjeldahl flasks. One-fifth of a gram of selenium was added to each flask of five of the batches, and 1 gm. of copper sulphate was added to each of the remainder. 7 gm. of sodium sulphate and 25 ml. of concentrated sulphuric acid were then added to all the flasks. The flasks were placed on the digestion stand with those containing copper sulphate and those containing selenium occupying alternate burners. The flasks were shaken at 3-min. intervals, and after each shaking were moved one place to the right, the flask at the end of the row being moved back to the other end. In this way it was hoped to eliminate variations due to the different heating powers of different burners. The mean total nitrogen figures are given in Table I, together with their standard errors, the digestion periods and the average times taken by the different batches to clear.

Table I. *Total nitrogen: percentage of oven-dried material*

Digestion period min.	Selenium			Copper sulphate		
	Means of four deter- minations	Standard error	Time (min.) taken to clear	Means of four deter- minations	Standard error	Time (min.) taken to clear
15	2.07	0.013	Did not	1.92	0.012	Did not
30	2.13	0.000	15	2.06	0.000	Did not
60	2.16	0.005	17	2.10	0.002	43
150	2.18	0.002	16	2.15	0.006	39
240	2.18	0.002	16	2.18	0.007	43
300	—	—	—	2.16	0.006	45

A maximum value for total nitrogen was obtained between the 60- and 150-min. periods with selenium, and between the 150- and 240-min. periods with copper sulphate. The copper sulphate figures were significantly ( $P < 0.05$ ) lower than the selenium figures until the 240-min. period, at which there was no significant difference between them. Hence, selenium not only cleared the contents of the digestion flask much more rapidly than copper sulphate, but it also accelerated the conversion of the nitrogen into ammonium sulphate. Both with selenium and with copper sulphate this conversion was by no means completed as soon as clearing occurred. The low value for the 300-min. period confirms Hibbard's (17) conclusion that a loss of ammonia may be brought about by heating after the bulk of the acid has boiled away.

It may be argued that the advantage of using selenium instead of copper sulphate is offset by the extra cost of this reagent. Precise calculation, however, showed that, with the petrol gas generator described above, it was cheaper to use selenium, as its extra cost was considerably less than the extra cost of the gas consumed in the longer digestion periods required with copper sulphate.

*Time required to complete the conversion of nitrogen  
with selenium as catalyst*

A further test, on three samples of grass, was made to determine more exactly the length of the digestion required when selenium is used as a catalyst. The lengths of the digestion periods, the numbers of determinations and the mean results of the analyses are given in Table II.

Table II. *Total nitrogen: percentage of oven-dried material*

Digestion periods in hours after clearing							
	No. of deter- minations	Stopped when clear	$\frac{1}{2}$	1	2	3	4
Sample I	4	1.32	1.31	1.35	—	1.36	—
Standard error		0.000	0.005	0.007	—	0.006	—
Sample II	6	3.00	—	3.04	3.06	3.08	3.07
Standard error		0.006	—	0.004	0.009	0.008	0.005
Sample III	6	2.88	—	2.94	2.96	2.97	2.97
Standard error		0.005	—	0.002	0.007	0.004	0.002

In every case a significant increase in the value for total nitrogen was obtained by digesting for an extra hour after clearing. A second hour gave further though smaller increases, and results apparently became constant between the 2- and 3-hour periods. This evidence, com-

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bined with that of the previous investigation, indicates that a digestion period of approximately  $2\frac{1}{2}$  hours is required to complete the conversion of nitrogen in grass to ammonium sulphate, when selenium is used as catalyst. This period might perhaps be shortened by stronger heating.

### *Length of the digestion period required with soils using selenium and copper sulphate as catalysts*

Three soils were selected for the experiment, soil A being an arable soil from the Canary Islands, and soils B and C meadow soils from Jealott's Hill Farm. These soils, according to the diphenylamine test, contained only a slight trace of nitrate and nitrite. They were air dried and ground to pass a 1-mm. sieve.

Owing to the amount of suspended matter that is always present when soils are being digested, it is doubtful whether the word "clear" may be used legitimately with regard to soil analysis. But there is a very definite point, where the digested matter ceases to lose colour, which is analogous to the clearing of less silicious materials. The word "clear" has, therefore, been retained to describe this point for lack of a better term.

10-gm. portions of soil were weighed into 500-ml. Kjeldahl flasks, to which were added 50 ml. water, 40 ml. concentrated sulphuric acid and 10 gm. potassium sulphate. One-fifth of a gram of selenium or 1 gm. of copper sulphate was used as a catalyst. Digestion periods, varying from when the contents of the flask became clear to 32 hours in addition to this period, were compared. Six replicate portions were digested with selenium, and six with copper sulphate for each period. Extra sulphuric acid was added to replace losses due to evaporation during the long digestion periods. The mean figures for each period are given in Table III.

In every case considerably higher figures were obtained when digestion was continued after the contents of the flask had become clear. In two cases the selenium figures increased for 3 hours, and in one case for 6 hours; all then remained constant up to the 32-hour period. In every case the copper sulphate figures showed a steady increase for 24 hours, after which they became constant at the same value as that reached more quickly by the selenium figures.

It was concluded that, after the contents of the flask had become clear, the digestion must be continued for a period of at least 3 hours with selenium, and for about 24 hours with copper sulphate, with these soils, if all the nitrogen present was to be converted to ammonium sulphate.

Table III. *Total nitrogen: percentage of air-dried soil  
(means of six determinations)*

Period	Digestion period in hours after becoming clear									
	Clear	+1½	+3	+4½	+6	+7½	+9	+18	+24	+32
Soil A:										
Selenium as catalyst	0.215	0.229	0.233	0.234	0.232	0.235	0.234	0.236	0.235	0.232
Standard error	0.0015	0.0008	0.0019	0.0012	0.0008	0.0016	0.0014	0.0007	0.0012	0.0020
Copper sulphate as catalyst	0.206	0.219	0.222	0.223	0.221	0.226	0.225	0.231	0.234	0.233
Standard error	0.0013	0.0024	0.0016	0.0029	0.0028	0.0007	0.0021	0.0012	0.0010	0.0014
Soil B:										
Selenium as catalyst	0.233	—	0.239	—	0.243	—	0.244	0.244	0.242	0.244
Standard error	0.0005	—	0.0008	—	0.0008	—	0.0007	0.0005	0.0003	0.0010
Copper sulphate as catalyst	0.230	—	0.237	—	0.239	—	0.239	0.240	0.243	0.244
Standard error	0.0009	—	0.0007	—	0.0002	—	0.0013	0.0008	0.0007	0.0004
Soil C:										
Selenium as catalyst	0.249	—	0.259	—	0.263	—	0.263	0.264	0.263	0.264
Standard error	0.0006	—	0.0013	—	0.0008	—	0.0008	0.0011	0.0010	0.0009
Copper sulphate as catalyst	0.245	—	0.255	—	0.255	—	0.257	0.261	0.261	0.265
Standard error	0.0006	—	0.0006	—	0.0009	—	0.0011	0.0013	0.0006	0.0004

There is, of course, the possibility that losses of ammonia took place during the long digestion periods which were balanced, in the case of selenium, and more than balanced with copper sulphate, by the conversion of the more difficultly decomposed nitrogenous compounds to ammonium sulphate. Hibbard's (17) conclusion, however, was that losses occur when the level of the acid in the flask becomes very low. Care was taken to see that this did not happen, and it seems probable that such losses did not take place.

The saving in cost of gas through the shorter digestion period with selenium, which has already been referred to in connection with the analysis of grass, is much greater in the case of soils, owing to the greater disparity between the times of digestion needed with the two catalysts.

*The inclusion of nitrate nitrogen in the determination  
when digesting soil in the presence of water*

The most usual modification of the Kjeldahl method for the inclusion of nitrate nitrogen in the determination depends upon the addition of an organic reagent to convert the nitric acid, liberated by the sulphuric acid, to a nitro compound, which is subsequently reduced to an amino compound by a second reagent. The principle of the modification was first suggested by Jodlbauer (18), who used phenolsulphonic acid and zinc. Later Scovell (19) and Frear (20) substituted salicylic acid and sodium thiosulphate. Whether phenol or salicylic acid is used, however, the

necessary nitration will not take place in the presence of more than traces of water. The only satisfactory way in which the salicylic acid modification could be combined with Bal's modification, using added water, appeared to be to reduce the nitrate before adding the water.

Another modification, first suggested by Ulsch<sup>(16)</sup> and not infrequently used to fix nitrate, utilises reduced iron powder. The iron is mixed with the substance, concentrated sulphuric acid is added, and digestion proceeded with in the usual way. The reaction is, however, so violent when sulphuric acid is added to the soil-water mixture (Bal) and the iron, that it is necessary to dilute the acid first and to add it after cooling.

The salicylic acid method, with and without water, and the reduced iron method were tested by making determinations after addition of known amounts of nitrate to a soil sample. A sample of fairly heavy soil which was free from nitrate and which had been air-dried and ground to pass a 1-mm. sieve was used. 10-gm. portions were weighed out for each determination and, when nitrate was added, 5 ml. of a standard potassium nitrate solution were pipetted on to them. The following methods of analysis were then tried:

(a) 50 ml. of water were first added to the soil and digestion carried out in the usual way.

(b) 1 gm. of salicylic acid was mixed with the soil and 40 ml. of sulphuric acid were added, followed by 5 gm. of sodium thiosulphate. Digestion was proceeded with in the usual way, but without the addition of water.

(c) The same method was used as with (b) but with 50 ml. of water added *after* the reaction following the addition of sodium thiosulphate was completed.

(d) 5 gm. of reduced iron powder were intimately mixed with the soil, and 90 ml. of cold dilute sulphuric acid, containing 40 ml. of concentrated sulphuric acid, were added and digestion carried out in the usual way.

In all cases selenium was used as a catalyst, and digestion was continued for 3 hours after the contents of the flasks had cleared. Six replicates without and six with added nitrate were analysed by each method. The average results are given in Table IV.

When no nitrate fixing reagents were added, (a), there was apparently a complete loss of the added nitrate (the slight apparent recovery shown in the table being within the limits of error). The effect of Bal's modification was illustrated by the fact that significantly lower values for total

nitrogen were obtained when no water was added. The recovery of nitrate with the use of salicylic acid followed by the addition of water was satisfactory, but that with reduced iron was disappointing in view of the work of other investigators (21).

Table IV

Method	% N in soil	% N in soil + added nitrate	Nitrate recovered (as % N)	Nitrate added (as % N)
(a) Bal's modification with no nitrate fixing reagents	0.267	0.269	0.002	0.014
Standard error	0.0012	0.0016		
(b) Salicylic acid modification, no water	0.254	0.272	0.018	0.014
Standard error	0.0031	0.0021		
(c) Salicylic acid modification + water (water added after reduction of nitrate)	0.266	0.281	0.015	0.014
Standard error	0.0004	0.0009		
(d) Reduced iron modification + water	0.265	0.274	0.009	0.014
Standard error	0.0022	0.0013		

The reduced iron used, however, was not very finely ground, and it was thought that this might account for its weak action. A further test was therefore made using the reduced iron supplied by Messrs Schering-Kahlbaum of Berlin, as this reagent was very much more finely ground. A recovery of 105 per cent. of added nitrate was found with the Schering-Kahlbaum reagent, and of 82 per cent. with the reduced iron used in the previous investigation.

These results show that a quantitative recovery of added nitrate can be obtained if a suitable preparation of reduced iron powder is used, and indicate that the unsatisfactory results first obtained were probably due to the use of insufficiently finely powdered material.

#### SUMMARY AND CONCLUSIONS

(a) A comparison was made between the catalytic efficiency of selenium and of copper sulphate in the Kjeldahl method as applied to the analysis of grass. The same value for total nitrogen was eventually obtained by both methods, but with selenium it was reached some 90 min. earlier than with copper sulphate.

(b) The clearing of the digested matter was no criterion for deciding whether all the nitrogen in grass had been converted to ammonium sulphate, maximum values not being obtained with selenium until between 2 and 3 hours afterwards.

(c) The catalysts were compared for three samples of soil with digestion periods ranging from when the digested material first cleared to



32 hours afterwards. The selenium figures showed an increase for the first 3 hours, and then remained constant up to the 32-hour period. The copper sulphate figures, however, showed a steady increase for the first 24 hours, after which they became constant at the same values as those reached earlier by the selenium figures.

(d) The salicylic acid modification for including nitrate nitrogen in the Kjeldahl method, as applied to soils, can be combined with Bal's "added water" modification, by first adding the reagents for converting the nitrate nitrogen to an amino compound, and adding the water after the reaction was complete. Ulsch's reduced iron method was also found to give satisfactory results, provided the reduced iron was sufficiently finely ground.

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# THE EFFECTS OF AMMONIUM AND NITRATE NITROGEN ON THE GROWTH OF PERENNIAL RYE GRASS<sup>1</sup>

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## INTRODUCTION

IN recent years considerable attention has been given to the nitrogenous manuring of grassland, and in order that nitrogenous fertilisers may be used to best advantage it is important to know how pasture plants react to different forms of nitrogen. This in effect resolves itself largely into a comparison of the effects of ammonium and nitrate nitrogen.

It is generally assumed that most plants obtain their nitrogen largely as nitrate, but there is ample evidence proving that ammonia is often directly absorbed by grasses<sup>(1, 2, 3, 4)</sup>. In particular, Richardson<sup>(1)</sup> has shown that, under certain conditions, grasses absorb ammonia rapidly and make good growth as a result. There is, however, no evidence of the relative effects, on growth and yield, of nitrogen absorbed as ammonia or as nitrate from soils of pH 6–7, at which the assimilation of ammonia would, according to the literature on water culture experiments, be expected to be at a maximum.

Whilst the results of water culture experiments are of considerable value, the conditions in such experiments differ very markedly from those in soil and any results, to be of practical significance, must be obtained under conditions approaching as closely as possible to those obtaining in the field. Unfortunately, the interpretation of results of experiments conducted in soil to compare the effects of ammonia and nitrate on growth is extremely difficult since nitrification usually occurs rapidly at that pH (6–7) which, as already stated, is optimum for assimilation of ammonia. Work conducted at Jealott's Hill<sup>(4)</sup> has, however, shown that grasses can absorb added ammonium and nitrate nitrogen when the soil temperature is so low that ammonification and nitrification are almost

<sup>1</sup> Being an abridged form of part of a "Thesis approved for the Degree of Doctor of Philosophy in the University of London".

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negligible, but growth of herbage only occurs when the temperature rises; as soon as this happens, ammonification and nitrification commence. Whilst, under these conditions, grass can be made to absorb relatively large amounts of ammonia, an appreciable amount of nitrate may also be absorbed during the period when the yield of grass is increasing.

For a critical comparison of the effects of ammonia and nitrate some means of preventing nitrification is necessary. Richardson<sup>(1)</sup> tried dicyanodiamide in the field at a rate corresponding to 0.2 cwt. N per acre, but found that nitrification was not completely inhibited; the use of dicyanodiamide is also open to the objection that it may adversely affect plant growth. Whilst the use of sterilised or partially sterilised soil would obviate difficulties due to nitrification, soil thus treated contains very large amounts of ammonia so that, while the effect of ammonia on growth could be studied, the effect of added nitrate could not be dissociated from that of the ammonia present; small amounts of nitrate might also be present in sterilised soil. The use of a medium consisting of calcium bentonite and sand, as described by Greenhill and Page<sup>(5)</sup>, would avoid the difficulties outlined above, since it contains practically no nitrogen or organic matter. Most of the ammonia would be held in an exchangeable form. Whilst any conclusions drawn from experiments conducted in this medium will not be unconditionally applicable under field conditions, the medium affords a convenient method of testing the relative effects of ammonia and nitrate under conditions less far removed from those obtaining in the field than does the only simple alternative, water cultures.

The experiments described below were, therefore, conducted in a medium of calcium bentonite and sand. No attempt was made to keep the medium sterile in order to prevent nitrification; however, samples of the medium taken from some of the pots after the experiments were completed, and incubated in the laboratory at 20 per cent. moisture content and 25° C., showed no nitrification of added ammonium sulphate or urea (100 p.p.m. N) after 3 weeks. Moreover, samples taken during the course of the experiment and tested with diphenylamine showed complete absence of nitrite and nitrate. Whilst this does not prove absolutely conclusively that nitrification did not occur, it does indicate that nitrate formation was not rapid, and that probably it did not occur to any appreciable extent.

## OUTLINE OF EXPERIMENTS

In the experiments described below the effects of ammonium sulphate, calcium nitrate and sodium nitrate (all supplying 0.75 gm. N per pot) on the growth of indigenous perennial rye grass were studied. Each manurial treatment was divided into three series cut at different stages of growth:

A. Short. Two cuts at 3 in. followed by one cut at  $4\frac{1}{2}$  in.

B. Medium. Two cuts at  $4\frac{1}{2}$  in.

C. Long. One cut at 6 in. followed by one cut at 4 in.

The last cut in all three series was taken when the grass was beginning to show signs of nitrogen deficiency. The manurial treatments were replicated eight times in series A and B and sixteen times in series C.

A control treatment (0.1 gm. N per pot) was also included; the results with this treatment are not given here, as the yields were extremely low and practically no growth occurred after cutting once.

## EXPERIMENTAL PROCEDURE

The pots (10 in. diameter  $\times$   $5\frac{1}{2}$  in. deep) were filled, in the same way as described by Greenhill and Page(5), with a mixture of 94 per cent. sand and 6 per cent. calcium bentonite. The moisture content of the medium was brought to 15 per cent. by adding well water. This preliminary work was completed by March 22, 1933. The pots were sown with 0.6 gm. indigenous perennial rye grass (about 380 seeds) on March 23, and the seed was covered with a shallow layer (9 oz.) of coarse sand to prevent caking. The surface was then covered with a single layer of filter paper, and water was added through this to prevent washing the seeds up to the surface. The filter papers were removed when germination was complete. The moisture content of the medium was maintained at 15 per cent. by weighing the pots frequently and adding distilled water as required.

In taking the cuts in the experiment proper, the grass was cut back to within  $\frac{1}{2}$  in. of pot level. The produce from each pot was dried at 98° C. and ground to pass a 1/64 in. sieve. The percentage nitrogen content of the ground material was determined by the Kjeldahl method modified to allow for the presence of nitrate by the addition of salicylic acid and sodium thiosulphate. The percentage  $P_2O_5$  content of the material was estimated by ashing in the presence of magnesium nitrate, extracting with dilute  $H_2SO_4$  and determining phosphorus in the extract by the Fiske-Subarrow method(6).

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### ESTABLISHMENT PERIOD

Germination was completed by about April 11, on which date the mean percentage germination was about 70. The grass was allowed from March 23 until June 15 to establish itself. During this period 0.25 gm. N, 0.27 gm.  $P_2O_5$  and 1.0 gm.  $K_2O$  were applied in solution by means of a fine jet to each pot. The grass was cut to within 1 in. of pot level on June 7. On June 15 the grass was cut to within  $\frac{1}{2}$  in. of pot level; it was obvious from its appearance that the grass was beginning to suffer from lack of nitrogen at this date.

### EXPERIMENTAL PERIOD

The experiment proper was started on June 16 when the nitrogenous fertilisers were applied in solution at a rate corresponding to 0.75 gm. N per pot. One day after applying the nitrogenous materials, the cut tips of the leaves in the ammonium sulphate treatment showed a white solid exudate similar to that described by Greenhill and Chibnall(3). On June 19 a "minor element mixture" (Mg, Fe and Mn) was applied, not because any deficiency of these elements was suspected, but merely as a safeguard. More  $P_2O_5$  and  $K_2O$  and "minor elements" were applied on June 18-20. Full details of the nutrients applied during the establishment and experimental periods are given in Appendix I. A summary of the salient features is given in Table I.

Table I

Date	Series		
	A (short)	B (medium)	C (long)
June 16	0.75 gm. N per pot	0.75 gm. N per pot	0.75 gm. N per pot
" 19	Minor elements	Minor elements	Minor elements
" 26	1st cut (3 in.)	—	—
July 6	2nd cut (3 in.)	—	—
" 7	—	1st cut ( $4\frac{1}{2}$ in.)	—
" 17	—	—	1st cut (6 in.)
" 18-20	0.5 gm. $P_2O_5$ per pot 1.0 gm. $K_2O$ per pot	0.5 gm. $P_2O_5$ per pot 1.0 gm. $K_2O$ per pot	0.5 gm. $P_2O_5$ per pot 1.0 gm. $K_2O$ per pot
" 21	Minor elements	Minor elements	Minor elements
Aug. 18	Final cut ( $4\frac{1}{2}$ in.)	Final cut ( $4\frac{1}{2}$ in.)	—
" 19	—	—	Final cut (4 in.)

### RESULTS

Since the results with calcium nitrate and sodium nitrate were, for all practical purposes, identical, they are not quoted separately, the average results with these two treatments being given in Table II.

Table II

	A series (short)				B series (medium)			C series (long)			Average of totals
	1st cut	2nd cut	Final cut	Total	1st cut	Final cut	Total	1st cut	Final cut	Total	
Dry matter, gm. per pot:											
Ammonia	3.63	3.24	7.61	14.48	8.67	8.47	17.14	16.03	5.97	22.00	17.87
Nitrate	3.42	2.54	7.46	13.42	7.07	8.18	15.24	12.38	5.99	18.36	15.67
Standard error of difference	0.198	0.199	0.557	0.626	0.451	0.477	0.780	0.578	0.205	0.667	—
Percentage N content of dry matter:											
Ammonia	5.59	4.09	2.08	3.40	3.70	1.93	2.81	2.68	2.15	2.54	2.85
Nitrate	5.04	4.26	2.31	3.36	3.70	2.16	2.85	2.76	2.38	2.63	2.91
Standard error of difference	0.088	0.074	0.052	—	0.065	0.054	—	0.036	0.036	—	—
Uptake of N, gm. per pot:											
Ammonia	0.2025	0.1322	0.1569	0.4916	0.3194	0.1618	0.4812	0.4296	0.1281	0.5577	0.5101
Nitrate	0.1716	0.1074	0.1721	0.4509	0.2588	0.1764	0.4351	0.3404	0.1423	0.4827	0.4562
Standard error of difference	0.0096	0.0075	0.0111	0.0146	0.0145	0.0101	0.0177	0.0142	0.0048	0.0145	—

Ammonium sulphate gave significantly greater total yields of dry matter than did calcium and sodium nitrates at all three heights of cutting; the differences between forms of nitrogen occurred almost entirely in the early cuts. The percentage nitrogen content was roughly inversely related to yield of dry matter and, except in the first short cut, was rather higher with added nitrate than with added ammonia. The total nitrogen uptake was greater with ammonia than with nitrate; the difference in favour of ammonia occurred in the early cuts, whilst in the final cuts the uptake with nitrate was greater than that with ammonia. The results show, therefore, a more rapid and greater uptake of ammonia than of nitrate.

#### DISCUSSION

Before it can be concluded that the greater rate of, and total, uptake of nitrogen with ammonium sulphate than with calcium nitrate and sodium nitrate were directly due to an intrinsically greater "availability" of ammonia than of nitrate, it is essential to consider other possible causes of this difference. The most obvious alternative explanations are that the nitrate treatments did not supply enough sulphate, or that there was an interaction between form of nitrogen and availability of phosphate.

Regarding the possibility of sulphate deficiency, the medium itself contained 0.03 per cent. water-soluble  $\text{SO}_3$  (equivalent to 3.4 gm.  $\text{SO}_3$  per pot), the well water applied at the start of the experiment supplied 0.275 gm.  $\text{SO}_3$  per pot, and the potassium sulphate added supplied

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0.25 gm.  $\text{SO}_3$ , excluding materials applied July 18. It is obvious that there was no deficiency of sulphate after this date. Although it seemed fairly obvious that this amount of sulphate was more than sufficient to supply the needs of the crop, to clinch this matter the sulphate content of the produce from some of the first C cuts, in which the difference in yield with ammonia and nitrate was greatest, was determined by Aitken's modification of the Benedict and Denis method (7). The percentage sulphate content of the dry matter was fairly high, and was almost the same with ammonium sulphate (0.784 per cent.  $\text{SO}_3$ ) as with calcium nitrate (0.773 per cent.  $\text{SO}_3$ ).

When the experiments were started there was little information available regarding the amount of phosphate necessary for good growth beyond the fact that very high recoveries of phosphate appeared to be possible with the growth medium used. The amount of  $\text{P}_2\text{O}_5$  added per pot (0.27 gm.) together with that in the medium (0.5625 gm. citric acid-soluble  $\text{P}_2\text{O}_5$ ) appeared to be sufficient to avoid phosphate deficiency at a nitrogen level of 1 gm. The phosphate content of the herbage from the second short and first medium cuts was, however, found to be low in comparison with figures quoted by Fagan (8) for perennial rye grass. It is possible that the figures quoted by Fagan may have involved a "luxury" consumption of phosphate, but as experience gained while the experiments were in progress indicated that considerably more soluble material could be added without harm, a further 0.5 gm.  $\text{P}_2\text{O}_5$  per pot was applied for the final cuts.

Table III. *Phosphate content*

		$\text{P}_2\text{O}_5$ : % of dry matter		$\text{P}_2\text{O}_5$ : gm. per pot	
		Ammonium sulphate	Sodium nitrate	Ammonium sulphate	Sodium nitrate
Short:	First cut	1.088	0.678	0.0390	0.0233
	Second cut	0.525	0.513	0.0180	0.0120
	Final cut	0.781	0.746	0.0600	0.0604
Medium:	First cut	0.488	0.378	0.0459	0.0258
	Final cut	0.645	0.688	0.0564	0.0500
Long:	First cut	0.307	0.273	0.0513	0.0345
	Final cut	0.654	0.575	0.0389	0.0365

From Table III it will be seen that the percentage  $\text{P}_2\text{O}_5$  content of the herbage and the  $\text{P}_2\text{O}_5$  uptake were higher throughout with ammonium sulphate than with sodium nitrate. It is probable that the same relationship holds good in the roots. Whether the greater  $\text{P}_2\text{O}_5$  uptake with ammonium sulphate resulted in increased growth cannot be answered

with certainty, but as the percentage  $P_2O_5$  content of the grass was low in comparison with figures generally accepted as normal, it seems likely that the greater yields with ammonium sulphate were due, at least in part, to a greater  $P_2O_5$  uptake.

The causes of the differences in  $P_2O_5$  uptake remain to be considered. It would appear that differences in physiological reaction between ammonium salts and nitrates might be reflected in differences in phosphate availability. The medium was somewhat alkaline ( $pH$  7.61) and highly buffered, and laboratory experiments indicate that the *maximum* change in  $pH$ , assuming penetration of the added nitrogenous fertilisers to a depth of only 2 in., would not be more than  $-0.5$  with ammonium sulphate and  $+0.3$  with sodium nitrate.

If the differences in  $P_2O_5$  content were entirely due to differences in physiological reaction of the fertilisers, then the difference in  $P_2O_5$  content in favour of ammonium sulphate might have been expected to increase with time, since the N content of the herbage in the first A cut was only 45 per cent. of that in the first C cut; actually the difference in  $P_2O_5$  content was only very slightly greater in the first C cut. Further, the  $P_2O_5$  figures were higher with ammonium sulphate in all the final cuts, despite the fact that a further 0.5 gm.  $P_2O_5$  had been applied per pot.

It is probable, therefore, that differences in physiological reaction of the nitrogenous fertilisers used were not entirely responsible for the differences in  $P_2O_5$  uptake, and it may be concluded that there are indications that uptake of ammonia was directly related to uptake of phosphate. This is in agreement with Pirschle's (9) results, which showed that in water cultures maintained at a constant  $pH$ , ammonia assimilation was associated with a greater phosphate uptake, and nitrate assimilation with a greater potash uptake in a number of crops.

It is, of course, possible that the differences between ammonia and nitrate found in the experiments described might not have occurred if the phosphate supply had been considerably greater. Further light should be thrown on the point by the results of experiments carried out by Greenhill (10) to study the relation of the uptake of phosphorus in relation to that of nitrogen.

#### SUMMARY

The results show clearly a more rapid and greater uptake of ammonium than of nitrate nitrogen by perennial rye grass grown in a sand-bentonite medium of  $pH$  7.61. The extent to which the extra



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uptake with added ammonia was reflected in increased yields was dependent upon the age of the grass.

Except in the very early stages, the percentage nitrogen content of the herbage was higher where nitrate nitrogen was applied than where ammonium nitrogen was applied. This indicates that any nitrate absorbed by the plant was less efficient in increasing yields than was ammonium nitrogen.

The percentage  $P_2O_5$  content of the grass was higher where the nitrogen was applied in the ammoniacal form than where it was applied as nitrate, and it appears that this greater  $P_2O_5$  uptake with ammonium nitrogen resulted in increased growth.

It is probable that differences in physiological reaction of the nitrogenous fertilisers were not entirely responsible for the differences in  $P_2O_5$  uptake, and there are indications that uptake of ammonia was directly related to uptake of phosphate.

### ACKNOWLEDGMENT

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### APPENDIX I

#### *Materials added per pot*

- Mar. 22. 1700 c.c. of water, containing 0.3315 gm.  $SO_4$ , 0.3349 gm. Cl, 0.0025 gm. free and albuminoid ammonia and 1.4688 gm. total solids.
- „ 23. Seed (0.6 gm.) sown.
- „ 27. 0.0835 gm. N (as calcium nitrate).

- Apr. 4. 0.085 gm.  $P_2O_5$  (as monocalcium phosphate).  
 „ 10. 0.0835 gm. N (as calcium nitrate). 0.25 gm.  $K_2O$  (as potassium sulphate).  
 „ 22. 0.0835 gm. N (as calcium nitrate). 0.085 gm.  $P_2O_5$  (as monocalcium phosphate).  
 „ 24. 0.25 gm.  $K_2O$  (as potassium sulphate).
- June 7. Grass cut back to 1 in.  
 „ 8. 0.1 gm.  $P_2O_5$  (as monocalcium phosphate).  
 „ 9. 0.5 gm.  $K_2O$  (as potassium sulphate).  
 „ 15. Grass cut back to 0.5 in.  
 „ 16. Experiment proper commenced. 0.75 gm. N applied per pot except to “controls”, which received 0.1 gm. N as  $Ca(NO_3)_2$ .  
 „ 19. 0.08 gm.  $MgO$  as  $MgSO_4$ . 0.01 gm. Fe as  $FeCl_3$ . 0.004 gm. Mn as  $MnSO_4$ .  
 „ 26. A series. 1st cut taken.
- July 6. A series. 2nd cut taken.  
 „ 7. B series. 1st cut taken.  
 „ 17. C series. 1st cut taken.  
 „ 18. 0.25 gm.  $P_2O_5$  as  $Na_2HPO_4$ . 0.60  $K_2O$  as  $KCl$ .  
 „ 19. 0.4 gm.  $K_2O$  as  $K_2SO_4$ . 1.80 gm.  $Na_2O$  as  $Na_2SO_4$ .  
 „ 20. 0.25 gm.  $P_2O_5$  as  $Na_2HPO_4$ . 1.20 gm.  $Na_2O$  as  $Na_2SO_4$ .  
 „ 21. 0.16 gm.  $MgO$  as  $MgSO_4$ . 0.02 gm. Fe as  $FeCl_3$ . 0.008 gm. Mn as  $MnSO_4$ .
- Aug. 18. A series. Final cut taken.  
 B series. Final cut taken.  
 „ 19. C series. Final cut taken.

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# THE RELATIVE IMPORTANCE OF PLOT VARIATION AND OF FIELD AND LABORATORY SAMPLING ERRORS IN SMALL PLOT PASTURE PRODUCTIVITY EXPERIMENTS

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## INTRODUCTION

SMALL plot experiments that have been carried out at Jealott's Hill on the productivity of pasture have usually consisted of treatments replicated on plots of about 1/200 acre. The herbage of each plot has been cut, collected into a heap, and the weight of fresh herbage obtained. From the heap a representative sample of from 2 to 5 lb. has been taken to the laboratory, and a subsample of 100–200 gm. taken for the determination of the percentage of dry matter in the fresh herbage. From the finely ground dried material the percentage of nitrogen (and other chemical constituents) has been determined on a representative sample of about 1 gm.

In this procedure the four sources of variation giving rise to experimental error are as follows:

- (1) The inevitable field variation among replicates, due to soil heterogeneity and other uncontrollable causes.
- (2) Sampling the heap of fresh herbage in the field.
- (3) Subsampling in the laboratory for dry-matter determination.
- (4) Sampling the ground dried material for nitrogen (and other chemical) determinations.

It was the object of the investigation described in this paper to determine the relative magnitudes of these different sources of error in a typical small plot pasture productivity experiment, with the view to assessing their significance in the technique of pasture plot experimentation.

## DESCRIPTION OF INVESTIGATION

The investigation was carried out on twelve plots of an experiment on the effect of rate and time of application of nitrogenous fertilisers. This experiment consists of six manurial treatments replicated in five random blocks, the herbage being cut each time it reaches the 4–5 in.

stage of growth. The twelve plots used for our test were the first two blocks, the date of cutting, the second time of cutting for this experiment during the year, being June 11, 1935. The botanical composition of the plots was noted, the dominant species, comprising about 66 per cent. of the herbage, being perennial rye grass; the yields of fresh herbage varied from about 25 to 45 lb. From each plot heap two samples of about 2 lb. were taken for laboratory examination, and from each of these samples two subsamples each of 200 gm. for the determination of dry-matter content by drying in the steam oven until constant in weight. The dried material of each of these four subsamples was ground separately (through a Christy and Norris 8 in. bag-delivery mill), and its nitrogen content determined in duplicate on 1 gm. portions by the standard Kjeldahl method using selenium powder as catalyst.

The plots were cut in fair weather and there was little external moisture present on the herbage. Growth was largely stemmy with many grasses in flower. The dry-matter content was in consequence fairly high (about 25 per cent.), with a low nitrogen content (about 1.75 per cent.).

#### INTERPRETATION OF RESULTS

The straightforward analysis of variance of the percentage dry matter and percentage nitrogen figures is given in Table I. The variation corresponding to blocks and treatment is irrelevant to this enquiry, and has been omitted. Further, the extra variation introduced by sampling the subsample of dried material for percentage nitrogen is so small that it is quite negligible, and was not worth considering in the analysis, which is therefore on a subsample basis both for percentage dry matter and percentage nitrogen.

Table I. *Analysis of variance*

	Degrees of freedom	Variance % dry matter	Variance % N
Among plots	5	6.4944	0.038993
Between samples	12	1.1277	0.007175
Between subsamples	24	0.2852	0.002004

Since there were two samples and hence four subsamples for each plot, we estimate the variances  $\sigma_1^2$ ,  $\sigma_2^2$  and  $\sigma_3^2$  corresponding to the variation between plots, samples and subsamples respectively, by noting that the three lines in the above analysis will represent

$$4\sigma_1^2 + 2\sigma_2^2 + \sigma_3^2, 2\sigma_2^2 + \sigma_3^2 \text{ and } \sigma_3^2.$$

The estimated variances so obtained are given in Table II.

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Table II. *Estimated variance*

Source	Variance		Variance as % total variance	
	% dry matter	% N	% dry matter	% N
Plots	1.342	0.00795	65	63
Sampling	0.422	0.00259	21	21
Subsampling	0.285	0.00200	14	16
Total	2.049	0.01254	100	100

Owing to the limitation in the number of samples that can be taken from any bulk of material, the figures are not direct estimates of the real variation among plots, etc., but are nevertheless estimates of the effective variances, in the sense that the variance of a value obtained from a plot by means of  $n$  samples and  $m$  subsamples is

$$\sigma_1^2 + \sigma_2^2/n + \sigma_3^2/m.$$

By means of this formula the comparatively slight extent to which increased sampling reduces the experimental error is illustrated in Table III. The last line of the table gives, of course, the standard errors actually found for the experiment.

Table III. *Estimated standard errors per plot*

Possible methods of sampling	Estimated s.e.		s.e. as % general mean	
	% dry matter	% N	% dry matter	% N
1 sample, 1 subsample	1.431	0.1120	5.69	6.39
1 sample, 2 subsamples	1.381	0.1074	5.49	6.13
2 samples, 1 subsample for each	1.302	0.1012	5.18	5.77
2 samples, 2 subsamples for each	1.275	0.0987	5.07	5.63

So far the sampling errors in percentage dry matter or percentage nitrogen have been considered only in relation to the variation in percentage dry matter or percentage nitrogen itself. Since these figures are usually intermediate measurements necessary for the estimation of yield of dry matter or yield of nitrogen, it is their relative importance in the estimation of these yield figures that has still to be considered.

The yield dry matter plot error depends on the fresh weights, the percentage dry matter, and the correlation  $\rho$  between fresh weight and percentage dry matter; if for convenience we express all the errors as percentages of the respective general means, the yield dry-matter error  $s$  is given approximately by the equation

$$s^2 = s_f^2 + s_p^2 + 2\rho s_f s_p,$$

$s_f$  and  $s_p$  standing for the fresh weight and percentage dry-matter errors respectively. It would be theoretically possible, if  $\rho$  were markedly negative, for the yield standard error  $s$  to be less than either the fresh weight or the percentage dry-matter error, but since the plot variation in fresh weight is usually more serious than any plot variation in percentage dry matter, and no *very strong* correlation is likely to exist, we may anticipate that  $s$  will certainly be greater than  $s_p$ . In this experiment there was no correlation apparent between the "error" components of fresh weight and percentage dry matter; the yield dry-matter plot error (including of course the error due to sampling) was 7.10 per cent. of the general mean as compared with 5.07 per cent. for percentage dry matter. The sampling errors will remain approximately the same percentages as before, and consequently are of even less importance when referred to the actual yields of dry matter.

A similar argument holds for percentage nitrogen sampling errors in relation to variation in yields of dry matter and percentage nitrogen.

#### DISCUSSION

In considering the results obtained, it should be remembered that, since treatments were part of the experiment, the estimate of plot error obtained from the analysis of variance is based on only five degrees of freedom, and rather inaccurately determined. Further, the grass contained little external moisture, and sampling errors are likely to be higher under wet conditions. The results appear, however, sufficiently representative of the variability usually met with to form a basis for general conclusions. Possible bias in sampling has been ignored, it is believed justifiably, since the difficult problem of sampling from heaps that have developed systematic variability does not arise here.

Evidently little advantage is gained by taking either duplicate samples in the field or duplicate subsamples in the laboratory. Of the two, duplicate sampling in the field is more important. The extra gain obtained by determining percentage nitrogen in duplicate from a subsample has already been dismissed as negligible. The chief justification of duplicate determinations of either percentage dry matter or percentage nitrogen is that they serve as a check against the possible return of an erroneous figure. Since the major part of the variation is caused by differences between plots, the number, uniformity and size of these plots is of most importance; thus any material reduction in our experimental error can be expected only by reducing this plot variation—by, for example, an increase in the number of replications in the field.

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### SUMMARY

An investigation of the relative importance in small plot pasture productivity experiments, of plot variations and of field and laboratory errors in sampling and subsampling for percentage dry matter and percentage nitrogen figures, revealed little advantage from duplicate sampling or subsampling in reducing the experimental error, which was due mainly to plot variation.

The effect of sampling errors was of even less importance in the estimation of the actual yields of dry matter or of nitrogen.

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# THE GROWTH OF CLOVER IN THE PRESENCE OF AMMONIUM SULPHATE

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It has long been held that the treatment of grassland with nitrogenous fertilizers, and especially with ammonium salts, reduces the clover content. This method has been applied also to the treatment of lawns and sports fields with a view to reducing the clover content of the turf. It has been suggested that the effect of the ammonium salts is to encourage the growth of the grass in competition with the clover, and also that the ammonium ions are specifically toxic to the clover plant. Since there is a growing volume of evidence that ammonium ions are, in fact, available to many plants and may be used by them directly, without nitrate-formation, it was thought desirable to examine the effect of the application of ammonium sulphate to clover plants grown in pots, where competition from grasses was not a factor in the consideration of the effect of manurial treatment.

In the first experiment Alsike clover seed was sown in sixteen pots each containing between 1 and 2 kg. of potting soil. The seed was sown in all pots on August 2, 1934. At the same time, the pots were grouped into four sets each of four pots. To the first set was added 1 gm. of ammonium sulphate per pot, to the second 0.5 gm. of ammonium sulphate, to the third 0.25 gm. of ammonium sulphate and the fourth was kept as a control. During the next few days the seeds germinated and the seedlings were thinned out as soon as the first foliage leaf appeared. Five plants were left in each pot, but further seeds germinated later. On September 20 another dressing of ammonium sulphate was given, each pot receiving the same dressing on this as on the previous occasion. This and all subsequent dressings were given in solution to ensure that an unduly high concentration of the sulphate did not become available to a single plant. The treatments were repeated in 1934 on these dates, viz. October 4, 18, November 1, 15, 29, December 13. In October the plants were taken into a cold glasshouse to prevent the washing of the soil in the heavy rains and to prevent damage to the foliage by wind. From the beginning of the experiment the pots had been placed in



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earthenware saucers to reduce to a minimum the loss of salts by leaching. The treatments were continued on January 17, 31, February 7, 21, March 14, 28, April 11, 25, May 9, 16, 30, June 13, July 4, 18, August 1, 15, 1935. Thus by the end of the experiment the "1 gm." series had received altogether 24 gm. of ammonium sulphate per pot. After May 9, the pots were again placed outside and the plants came into flower about the beginning of June. ✓ At no time in the experiment was there any evidence that the ammonium sulphate had any but a slight beneficial effect on the clover plants, as the plants receiving sulphate, especially at the highest level, were even darker green than the controls. All the plants grew very well indeed, and flowered profusely: in the early stages of the experiment the ammonium sulphate plants were clearly larger and better grown than the controls, but this difference disappeared later.

On December 6, 1934, after the experiment had been running for five months, and a week after the seventh application of sulphate, four of the pots, one from each treatment, were taken for analysis. A few root nodules were observed in all the pots. The plants, both tops and roots, and the soils were weighed and analysed (Tables I and II). Ammonia and nitrate nitrogen were determined in the fresh soils by Carsten Olsen's method, the sum of these two being shown in Table I as "mineral nitrogen". The "extra mineral nitrogen", obtained by subtracting that in the control pot from the mineral nitrogen in the treated pots, shows how much of the nitrogen added as ammonium sulphate was still present in the soils. Considerable amounts of nitrate and also, in the "1 gm." pot, a large amount of ammonia were found in the treated soils. Since over half of this ammonia was water-soluble, ammonium ions were doubtless present.

On May 29, 1935, the plants from two pots of each treatment were cut for an analysis of the foliage. The plants had now flowered and were

Table I. *Analysis of soils, December 6, 1934*

Treatment gm. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		Weight of moist soil kg.	Reac- tion pH	Dry matter %	Ammonia nitrogen mg./kg. dry soil	Nitrate nitrogen mg./kg. dry soil	Mineral nitrogen gm./pot	Extra mineral nitrogen gm./pot
Each dressing	Total added							
0.0	0.0	1.28	7.1	76.5	7.6	50.7	0.06	—
0.25	1.75	1.45	6.3	66.7	14.6	394.0	0.39	0.33
0.50	3.50	1.16	6.0	74.9	71.8	378.0	0.39	0.33
1.00	7.00	1.56	5.2	68.8	272.0	626.0	0.96	0.91
					(Water- soluble)			
					144.0			

Table II. *Analysis of plants, December 6, 1934*

Treatment gm. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		No. of plants found	Tops			Roots			Total nitrogen in plant gm./pot
Each dressing	Total added		Weight dry gm./pot	Nitrogen		Weight dry gm./pot	Nitrogen		
				%	gm./pot		%	gm./pot	
0.0	0.0	12	5.7	4.00	0.23	3.8	3.54	0.13	0.36
0.25	1.75	7	4.4	4.21	0.18	2.9	3.79	0.11	0.30
0.50	3.50	15	6.8	4.29	0.29	4.0	4.26	0.17	0.46
1.00	7.00	16	6.3	4.81	0.30	3.5	4.66	0.16	0.47

nearly mature. The nitrogen percentage on the dry matter varied between 3.11 and 3.47, but there was no significant difference between the treated and the controls (mean  $3.24 \pm 0.13$ ). There was further no significant difference between the treated and the controls as regards the amount of foliage, expressed as fresh weight or as dry (mean dry wt.  $45.9 \text{ gm.} \pm 3.6$ ).

Immediately after cutting, growth of fresh shoots took place and the second crop was flowering when it was harvested on August 6. All the pots were harvested on this date, practically a year after the experiment had first been set up. Little difference was found in the nitrogen content of the tops of the plants in the various treatments, the main difference being that the tops of the plants which had not been cut down earlier were more fibrous, and consequently had a lower percentage nitrogen content than had the others of the same group. There were no significant differences between treatments in yield (mean dry wt.  $39.6 \text{ gm.} \pm 2.5$ ) or in nitrogen content (mean 2.44 per cent.  $\pm 0.08$ ). The details for nitrogen are given in Table III, in which the (a) and (b) series were those cut on May 29. It will be noted that the nitrogen content of all the plants is lower than it was in the earlier analyses.

Table III. *Nitrogen content of foliage, August 6, 1935*

Treatment gm. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		Not cut on May 29 Nitrogen %	Cut on May 29	
Each dressing	Total added		(a) Nitrogen %	(b) Nitrogen %
0.0	0.0	2.00	2.49	2.61
0.25	5.75	2.33	2.46	2.63
0.50	11.50	2.14	2.70	2.42
1.00	23.00	2.29	2.61	2.59

The treatments were continued after the foliage samples had been taken until August 29, when another cut was taken and the experiment was abandoned, the soils being analysed. Just before the final analyses were made the weather had been exceptionally wet, and, as a conse-

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quence, some leaching had taken place from the pots. The final analyses are detailed in Table IV.

Table IV. *Analysis of soils, August 29, 1935*  
(average of three pots)

Treatment gm. $(\text{NH}_4)_2\text{SO}_4$		Soil pH	Ammonia nitrogen mg./kg. dry soil	Nitrate nitrogen mg./kg. dry soil	Mineral nitrogen gm./pot
Each dressing	Total added				
0.0	0.0	6.9	8.2	5.6	0.015
0.25	6.0	6.3	8.9	9.2	0.020
0.5	12.0	5.8	30.2	8.5	0.043
1.00	24.0	4.6	105.0	8.4	0.125

The root systems were all very well developed and a few nodules were present on all the plants. There were no significant differences in yield of foliage (mean dry wt. 5.6 gm.  $\pm$  1.2). The nitrogen content of the plants is shown in Table V; the nitrogen content with the 1 gm. treatment was significantly above the others.

Table V. *Nitrogen content of foliage, August 29, 1935*  
(average of three pots)

Treatment gm. $(\text{NH}_4)_2\text{SO}_4$		Total nitrogen % of dry matter
Each dressing	Total added	
0.0	0.0	3.73
0.25	6.0	3.68
0.50	12.0	3.73
1.00	24.0	4.50
S.E.		$\pm 0.18$

### EXPERIMENT WITH SAND CULTURES

A similar set of experiments was made with red clover grown in sand in glazed porcelain pots. This experiment was set up on May 21, 1935, when dressings of 0.0, 0.25, 0.50 and 1.00 gm. of ammonium sulphate were given to each set of pots. A basal dressing of Knop's solution (without nitrogen) was given to each pot on May 21, and again on June 18. The nitrogen treatments were continued on May 30, June 13, July 4, 18, August 1, 15, making in all seven applications of ammonium sulphate. This experiment also was stopped on August 29. The pots were kept in a glasshouse for the first six weeks and were then put out of doors. There was a spell of hot weather during July and the growth of all the plants was rather retarded but, in spite of this, on examination at the end of the experiment the roots were well developed, and a few large root nodules were present on all the plants. The plants receiving

the high sulphate dressings grew well, and while their leaves were on the whole narrower and darker green than those of the control plants, the amount of dry matter produced was about equal to that of the controls. The nitrogen content of the foliage was slightly greater in the plants with the high sulphate dressings, being on an average 2.93, 3.00, 3.36 and 3.32 per cent. of the dry matter in the 0, 0.25, 0.50 and 1 gm. sulphate pots respectively.

#### DISCUSSION AND CONCLUSIONS

A number of interesting points emerge from a consideration of the data obtained in this experiment. Some of these will be examined in more detail in later experiments. It does appear, however, as a general conclusion, that ammonium sulphate, as such, cannot be considered specifically toxic to Alsike or red clovers, even when applied in relatively enormous doses, *e.g.* 24 gm. in all per kg. of dry soil. In the early stages of growth the ammonium salt has a definitely stimulating effect on the plants, and at no stage does it have a retarding effect—the only difference in growth noticeable in the plant being the narrowing of the leaflets in the red clovers grown in sand with the highest sulphate treatments, and some reduction in the size of their roots.

These results harmonize with the conclusion reached by one of us (Richardson<sup>(2)</sup>) in an experiment with a number of nitrogenous treatments on mown grassland. He observed little direct injury to clovers from sulphate of ammonia, but found a significant negative correlation between the yield of herbage on all plots in the early part of the year and the growth of clover in July, and concluded that the chief factor in the repression of clover by sulphate of ammonia was simply competition with the extra growth of grass produced (see also Martin Jones<sup>(1)</sup>).

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# STUDIES ON *AZOTOBACTER* IN MALAYAN SOILS

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(With One Text-figure)

## INTRODUCTION

NON-SYMBIOTIC nitrogen fixation is confined to two groups of bacteria, the anaerobic group represented by the genus *Clostridium*, and the aerobic group represented by the genus *Azotobacter*. On the basis of conclusions, relating to the ecology of these organisms, which had been reached in Europe and in the United States, it was to be assumed that *Azotobacter* would be absent from the typical Malayan soils, whose hydrogen-ion concentration ranges between pH 5.5 and 4.5, but that *Clostridium* might possibly be present. Gainey<sup>(1)</sup> had ascertained that *Azotobacter* occurred only in soils whose hydrogen-ion concentration had a pH value of 6.0 or more, and his observations, confirmed by other workers, had received general acceptance. The position in regard to *Clostridium* was not quite so clear, but was thus summed up by Waksman<sup>(2)</sup>, p. 111: "The optimum reaction for the growth of *Cl. pastorianum* is pH 6.9 to 7.3, but it still develops well at pH 5.7...it can thus withstand a greater acidity than *Azotobacter*, whose limit is pH 6.0." These views were based exclusively on observations made on temperate soils, for no such work had been done in the tropics. Ashby<sup>(3)</sup> had reported the occurrence of *Azotobacter* in three types of Jamaican soil, Groenewege<sup>(4)</sup> had isolated this organism from several Javanese soils and Walton<sup>(5)</sup> had found it to be present in six Indian soils, but the hydrogen-ion concentration of the soils concerned was not known. These tropical studies had, however, preceded Gainey's work by some years and it was natural to conclude that had the necessary data been ascertained they would have substantiated his results. Fortunately (for otherwise it is doubtful whether the presence of the organism would have been determined) it was decided to examine the position with regard to *Azotobacter* in Malaya by the use of a technique which had been described by Winogradsky<sup>(6)</sup>, rather than employ the standard method introduced by Ashby<sup>(7)</sup>. Ashby's method involves the use of a liquid medium and

this, owing to the deficiency of oxygen in such an environment, provides conditions which are not at their optima for the growth of a vigorous aerobe. To overcome this fault, Winogradsky employed a solid medium the basis of which was silica-gel. Both media contained similar nutrients and mannitol as a source of energy.

#### PRELIMINARY OBSERVATIONS

The first soil to be investigated was a quartzite soil of pH 4.5. A slight modification of Winogradsky's technique had to be introduced for the large Petri dishes of 20 cm. diameter, which he recommended, were not available, and it was necessary to use the ordinary dishes of 9.5 cm. diameter. This entailed a reduction in the quantity of soil used as an inoculum. Two plates were prepared and each inoculated with 0.5 gm. of sieved soil. Daily observations were made, but both cultures were still sterile at the end of a fortnight. In the meanwhile a second soil had been submitted to examination. This was an organic alluvial soil of pH 4.6, and in this case eight plates containing the selective silica-gel medium were inoculated. Four days after inoculation a colourless gelatinous growth began to develop on one of the plates. Microscopic examination showed that this consisted of typical cells of *Azotobacter*. Four days later similar colonies had appeared on three more of the plates, and twelve days after were observed on the fifth plate. The other three plates remained sterile. While the second experiment was proceeding the two plates inoculated with the quartzite soil had been kept under observation, and some thirty days after inoculation a few colonies of *Azotobacter* began to develop on one of the pair.

It will be noticed that with both soils a remarkably long interval occurred before *Azotobacter* began to develop on some of the plates, and that, in each series, a proportion of the plates remained permanently sterile. By the use of the same technique it is found that the presence of *Azotobacter* in temperate soils can be demonstrated, by the development of colonies, within two to three days, and that commonly all the mannitol present on the plates has been consumed ten to twelve days after inoculation (6). The delayed appearance of an organism in culture has usually been regarded as evidence of its low concentration in the original inoculum, but, in the present instance, such an explanation seemed insufficient in view of the inconsistent behaviour of the eight cultures set up from the organic alluvial soil; for, although the eight inocula had been derived from a well-mixed sample, the lag phase had ranged, in this single series, from four days to infinity.

However, since *Azotobacter* had been found to be present in these soils, it seemed that it might be possible, by means of Winogradsky's technique, to undertake a comparative survey of the "nitrogen-fixing power" of the main soil types. As a preliminary experiment, three plates were inoculated with a cultivated quartzite soil whose hydrogen-ion concentration was pH 6.2 and thus within Gainey's limit. One plate was immediately treated with nitrogen-free  $\text{H}_2\text{SO}_4$ , and this acted as a control. The development of the organism was rapid, and the gain in nitrogen was estimated fourteen days after the plates had been inoculated, by which time all the mannitol had been consumed. That this was the case was shown by the fact that although one plate had received only half the charge of soil received by the other the amount of nitrogen fixed at the end of this period was approximately the same in both cases. An average of 7 mg. of nitrogen were fixed for every gram of mannitol consumed, showing that the "nitrogen-fixing power" of this quartzite soil, as determined by Winogradsky's method, was not far short of that recorded for fertile temperate soils. The bulk of the fixation was due to *Azotobacter*, but a few colonies of *Clostridium* developed on both plates under the *Azotobacter* films.

This experiment is unique in that the growth rate of the *Azotobacter* was comparable to the rate recorded for temperate soils, and as the pH value of the soil under examination was 6.2 it would appear to confirm Gainey's observations. But it will be seen that although the same soil was used in later experiments an analogous effect was never again obtained.

#### OBSERVATIONS WITH LIQUID MEDIA

Manipulative difficulties encountered in the course of the above experiment suggested that the development of a routine method, which was the ultimate object, could more easily be achieved by reverting to the standard method of Ashby. In the first trial of this method sixteen 350 c.c. Erlenmeyer flasks each containing 50 c.c. of Waksman's standard mannitol solution (2, p. 730) were inoculated with 1 gm. of the organic alluvial soil of pH 4.6 to which previous reference has been made. Two flasks were suitably treated and set aside as controls. Twelve days after inoculation the fourteen remaining flasks were still apparently sterile. As the soil with which they had been inoculated had already been shown to contain *Azotobacter*, it seemed only possible to account for this result by supposing that the composition of the medium was at fault. To determine whether this was the case, six of the flasks were re-inoculated

with a pure culture of *Azotobacter* derived from the former cultures of the same soil on Winogradsky's silica-gel medium. The flasks were inoculated in pairs, the amount of the inoculum being graded, the second and third pairs receiving respectively twice and three times the quantity of the first pair. Growth of *Azotobacter* eventually took place in all the flasks thus treated, but the rate of development of the organism appeared to bear no relation to the amount of inoculum added. Nevertheless it was evident that the batch of medium in use was capable of supporting growth, providing it were inoculated with a large number of the organisms. Since this suggested that the failure of the original inoculations might be due to the low concentration of *Azotobacter* in the soil, two of the eight remaining flasks, which had now remained sterile for forty-six days, were each re-inoculated with 2 gm. of the same soil, and at the same time three others were re-inoculated, respectively, with 1, 2 and 3 gm. of the cultivated quartzite soil of pH 6.2 which had been found to be comparatively rich in *Azotobacter*.

The effect of the addition of the cultivated quartzite soil was to initiate butyric fermentation, which was assumed to be due to *Clostridium*, for this organism was found to be abundant in the sediment at the base of the flasks. The time of appearance and rate of progress of this fermentation bore a definite relationship to the amount of soil added. The re-inoculation of the two flasks with 2 gm. of organic alluvial soil had no effect, but when the hydrogen-ion concentration of the medium in one was increased by the addition of *N*/10 hydrochloric acid butyric fermentation set in.

The experiment was abandoned sixty-nine days after its initiation, and at that date *Azotobacter* had failed to make an appearance in any flask other than the six which had been inoculated with a pure culture of the organism; butyric fermentation had been induced in three flasks by the addition of a soil of pH 6.2, and in one by an increase in hydrogen-ion concentration; the other four flasks remained sterile.

#### *Effect of a reduction in depth of media, and of larger inocula*

The complete failure of this trial of Ashby's method was disconcerting, and it was evident that no progress could be made until this unexpected result had been explained. The fact that *Azotobacter* had developed from both the soils when they had been plated on selective silica-gel suggested that the inability of the organism to initiate growth in the standard mannitol solution was associated with a deficiency of oxygen. An experiment was set up to investigate this point and to



re-examine the possibility that the failure was due to the scarcity of *Azotobacter* in the soil. Six 350 c.c. Erlenmeyer flasks each containing 50 c.c. of standard mannitol solution were inoculated in pairs with 1, 2 and 6 gm. respectively, of the cultivated quartzite soil of pH 6.2. Two 500 c.c. Winogradsky flasks also containing 50 c.c. of the nutrient solution were inoculated with 1 gm. of the soil. Owing to the shape and size of these latter flasks the depth of liquid was reduced to 3 mm. and oxygen supply thus improved. Three days after inoculation butyric fermentation was observed to be in action in the six Erlenmeyer flasks, being particularly vigorous in the pair which had received a 6 gm. inoculum, but was absent from the pair of Winogradsky flasks due, it was assumed, to effect of improved aeration on the anaerobic organism *Clostridium*. Three days later, when all the Erlenmeyer flasks were exhibiting active fermentation, this reaction was only just beginning to appear in one of the Winogradsky flasks, and was still absent from the other. The experiment was discontinued after three weeks, by which time fermentation had occurred in the remaining Winogradsky flask. *Azotobacter* had not been observed in any of the cultures, and thus it seemed evident that neither the amount of the inoculum nor the insufficiency of oxygen was the factor primarily responsible for the failure of the method.

*Influence of hydrogen-ion concentration and of calcium carbonate*

It was possible that the inhibitory factor was one connected with the constitution of the medium. In the original trial it was significant that, whereas butyric fermentation had been initiated by the addition to the flasks of a soil of pH 6.2, no effect was forthcoming when the re-inoculation was carried out with a soil of pH 4.6 until the reaction of the medium had been changed by the addition of *N*/10 hydrochloric acid. If it were supposed that the bacterial strains occurring in the acid and lime-deficient Malayan soils were either calciphobous or favoured an acid environment, then the failure of the method might well be due to the hydrogen-ion concentration of the medium or to the presence of calcium carbonate. In the preparation of the standard mannitol solution, which contains 0.5 per cent. calcium carbonate, the reaction is rendered neutral to phenolphthalein by the addition of *N*/10 NaOH. Media prepared in this way were found to have a pH value of 7.6 to 8.0, and, if either of the hypotheses regarding the ecology of local strains of soil bacteria were correct, such media would obviously provide a most unsatisfactory environment.

A mannitol solution was prepared according to Ashby's formula (7), with the exception that calcium carbonate was omitted. The medium was divided into eight lots and the hydrogen-ion concentration of each lot was adjusted, by means of standard HCl and NaOH, so that a series was obtained having the following range of pH values: 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0. The medium representative of each pH was transferred, in 50 c.c. lots, to pairs of sterile flasks, and the sixteen flasks thus obtained were each inoculated with 1 gm. of the cultivated quartzite soil. Butyric fermentation occurred in every flask with the exception of one of the pH 5.0 pair, which, owing to loss during adjustment, contained only half the normal quantity of medium, and was therefore better aerated.

The cultures were examined from time to time but no evidence of the development of a typical *Azotobacter* film was to be observed. Two months after inoculation, when the flasks were being subjected to a final examination before being discarded, it was noticed that the surface of the medium in the pH 5.0 flask, already mentioned, was covered with a colourless gelatinous film, and that a similar growth was present in all the other flasks, but in these was confined to the wall of the vessel at the position of contact of the surface of the liquid. Examination of stained smears of this growth showed that it consisted of *Azotobacter*-like organisms, some in particular bearing a resemblance to *Bacillus malabarensis*, an aerobic nitrogen-fixing organism described from South India, which is now regarded as a stage in the life-cycle of *Azotobacter*. The hydrogen-ion concentration of the media in the pH 4.5 and 8.0 flasks was determined at this time, and both were found to have attained a pH value of 3.6.

Providing, as was subsequently found to be the case, that the gelatinous growth consisted of normal *Azotobacter*, some progress had been made. For it had been established that this organism could be induced to develop from a soil inoculated into mannitol solutions which contained no calcium carbonate and whose original pH values ranged from 4.5 to 8.0, whereas the same soil had failed to yield the organism when inoculated, even in increased amounts, into a similar solution of pH 7.6 containing calcium carbonate. These observations could be interpreted by supposing that the strain of *Azotobacter* under study was calciphobous, but able to withstand a wide range of hydrogen-ion concentrations. Under the conditions of the experiment there was nothing to suggest that any one of the hydrogen-ion concentrations studied was more favourable than the other, for the more vigorous development in the single pH 5.0 flask could only be related to improved aeration, nevertheless this chance observation indicated that oxygen supply was a

factor of some importance. However, it was plain that the environmental conditions provided by the modifications of the media examined were far from the optimum, and the extraordinary lag phase of two months' duration had yet to be explained.

#### *Effect of aeration*

Some of the gelatinous growth from the walls of one of the pH 5.5 flasks was transferred to two tubes containing a mannitol solution of pH 7.5, to which no calcium carbonate had been added, and so arranged that a continuous stream of sterile air could be bubbled through. An uninoculated control tube was attached. Six days after the experiment was begun the solution in the inoculated tubes was seen to be assuming a turbid appearance, and examination of this liquid showed that it now contained a strong suspension of typical *Azotobacter* cells. This strain was subsequently isolated on nitrogen-free mannitol agar, on which, at pH 5.5, it grew vigorously, and was maintained in pure culture on this medium for about eighteen months. During this period a study was made of its morphology and cultural characteristics on various media. On mannitol agar containing nitrogen in the form of potassium nitrate or asparagin its growth was much retarded; on mannitol agar to which 0.5 per cent. calcium carbonate had been added vigour was hardly affected but a brick-red pigment was developed.

An improvement in the conditions of the air supply had markedly encouraged the reproduction of the organism in a liquid environment, so there was reason to believe that the provision of similar conditions might induce its more rapid development from the soil, when the latter was inoculated into some of the modifications of mannitol solution already examined. An aeration apparatus was set up consisting of tubes containing three modifications of mannitol solution: (a) without calcium carbonate, of pH 4.6, (b) without calcium carbonate, of pH 5.6, (c) containing 0.5 per cent. calcium carbonate, of pH 7.6. Each of the tubes was inoculated with 1 gm. of the cultivated quartzite soil, and a continuous stream of sterile air then passed through. After twenty days' aeration *Azotobacter* was found to have developed as a gelatinous film on the walls of the tubes containing the solutions of pH 4.6 and 5.6, but was not to be observed in the tube containing the solution, of pH 7.6, to which calcium carbonate had been added, but it made its appearance in this five days later.

It was clear therefore that, for the purpose of studying a local soil, Ashby's technique could be improved if provision were made for adequate aeration of the nutrient solution; for under such conditions the lag phase,

in the acid media, had been decreased by two-thirds, and *Azotobacter* had, for the first time, been induced to develop in standard mannitol solution after a lag of only twenty-five days. There was nothing to indicate that either one of the acid modifications provided a more suitable environment than the other, but once more there was evidence that the growth of the organism was inhibited by the presence of calcium carbonate.

### *Influence of other factors*

Although, perhaps, a means had been found of adapting Ashby's method for use under local conditions, it had not yet been explained why the conditions of aeration occurring in a liquid environment should be suitable for the development of *Azotobacter* from temperate soils but unsuitable in the case of Malayan soils. It was possible that the main effect of improved aeration was to counteract some unknown inhibitory factor associated with the composition of the medium. A search was made for this factor. Modifications of mannitol solution, containing varying concentrations of potassium dihydrogen phosphate, of minerals other than the phosphate, and of mannitol, were investigated with negative results. It had been reported by Olaru (8) that nitrogen-fixation by *Azotobacter* was stimulated in the presence of salts of manganese, the optimum concentration in mannitol solutions being 0.001–0.002 per cent., so observations were made on solutions containing manganese sulphate in both concentrations, but there was nothing to show that these additions had had any effect. A study of the influence of colloidal silica, suggested by Sohngen's work (9), was equally inconclusive.

### OBSERVATIONS WITH SOLID MEDIA

It seemed that the problem might be simplified were future observations to be carried out on solid media, thus avoiding the complications introduced by a liquid environment. A series of silica-gel plates containing three modifications of a mannitol medium, which differed in regard to their calcium content and hydrogen-ion concentration, were prepared. The basic medium resembled that used by Winogradsky and was as follows:

Distilled water	...	100.0 c.c.
KH <sub>2</sub> PO <sub>4</sub>	...	0.25 gm.
MgSO <sub>4</sub>	...	0.15 gm.
NaCl	...	0.15 gm.
FeCl <sub>3</sub>	...	0.001 gm.
MnSO <sub>4</sub>	...	0.001 gm.
Mannitol	...	10.0 gm.

Modification A contained 2.5 per cent. of calcium carbonate and had a hydrogen-ion concentration of  $pH$  6.0, modification B contained 0.075 per cent. of calcium chloride and had a  $pH$  value of 4.8, and modification C contained no calcium but had the same hydrogen concentration as modification B. Eight plates were prepared, four containing medium A, two containing medium B and two containing medium C. Each plate was inoculated with 0.25 gm. of a quartzite soil of  $pH$  5.6. Immediately after inoculation, two medium A plates were treated with nitrogen-free  $2N$   $H_2SO_4$  (to prevent loss of volatile, basic nitrogen compounds), dried out at  $60^\circ C$ . and set aside as controls. The cultures remained sterile for twelve days, when colonies began to develop on the pair of plates containing medium B. Three days later growth was apparent on all. A count of the colonies was made nineteen days after inoculation and gave the following figures:

Series	$pH$	Number of colonies		
		Plate 1	Plate 2	Mean
Medium A (2.5 % $CaCO_3$ )	6.0	11	12	11.5
Medium B (0.075 % $CaCl_2$ )	4.8	25	32	28.5
Medium C (no calcium)	4.8	25	31	28.0

From the above table it will be observed that the number of colonies which had developed on media B and C was approximately equal and more than double the number that had developed on medium A. This result not only appeared to provide conclusive evidence of the existence of an inhibitory factor introduced by the presence of calcium carbonate, but indicated that no calcium, or no more than the trace which might occur in the soil used as an inoculum or as an impurity in the medium, was necessary for the development of the strain of *Azotobacter* concerned.

A determination was made of the quantity of nitrogen which had been fixed in these cultures, and although, as will be seen from the table below, there was no close correlation between the figures thus obtained and the number of colonies, yet it was clear that fixation had taken place in an acid medium, in the absence of calcium carbonate, and in the absence of added calcium.

Series	$pH$	Nitrogen fixed, mg. Mean
Medium A (2.5 % $CaCO_3$ )	6.0	0.20
Medium B (0.075 % $CaCl_2$ )	4.8	0.35
Medium C (no calcium)	4.8	0.25

The experiment was now repeated, using in place of the former soil a quartzite soil of  $pH$  4.5, and at once the difficulties which had been encountered in the earlier work reappeared. While it is true that in the

experiment just described a lag of twelve days occurred before the first colony became visible, once the organism had started to develop, the rates of growth on duplicate plates closely approximated. But in the second experiment not only was the lag phase extended to thirty-nine days, but *Azotobacter* failed entirely to develop on the medium containing calcium carbonate and appeared only on one of each of the pairs of plates containing media B and C.

*Influence of various carbon compounds*

In the previous studies it had been found that the lag phase in liquid media could be shortened by improving the air supply, but it was suggested that this procedure merely assisted in reducing the inhibitory effect of a factor which was nevertheless still present and associated with the composition of the medium. Various attempts had been made to determine the nature of this factor, none of which had met with success. It was evident, however, that an extended lag phase could not be attributed in any way to defective aeration in the case of cultures on solid media, and must either be associated with some factor in the composition of the medium, or due to the low concentration of the organism in the soil. The two experiments just described indicated that calcium carbonate, although in itself an inhibitory factor, was not the only one, for a marked lag had occurred in its absence and even in the absence of calcium. It seemed possible that mannitol itself might be an unsuitable source of energy for the local strains of *Azotobacter*. Admittedly, in the work on temperate soils, mannitol had been found to be most satisfactory, but the suitability of certain other carbon compounds was at least worthy of investigation.

Using as a base a mineral solution of the same constitution as that used in the previously mentioned medium B, five nutrient solutions containing, respectively, mannitol, glucose, dextrin, starch and lactic acid were prepared<sup>1</sup>. The hydrogen-ion concentration of these solutions was determined and found to be as follows:

Mannitol medium	...	...	pH 4.8
Glucose medium	...	...	pH 4.8
Dextrin medium	...	...	pH 5.0
Starch medium	...	...	pH 4.8
Lactic acid medium	...	...	pH 2.0

<sup>1</sup> The mannitol, glucose, dextrin and lactic acid were present in 10 per cent. concentrations; the starch in a concentration of 5 per cent., a 10 per cent. gel being found too viscous to handle.

The lactic acid solution was divided into two lots, and the reaction of one adjusted to a pH value of 5.0 by means of standard NaOH. This sixth modification thus contained a mixture of lactic acid and sodium lactate.

Six pairs of silica-gel plates were prepared, each pair containing standard quantities of one of the six nutrient solutions. Two plates were set up as controls. Each of the fourteen plates was inoculated with 0.25 gm. of the cultivated quartzite soil. Immediately after inoculation

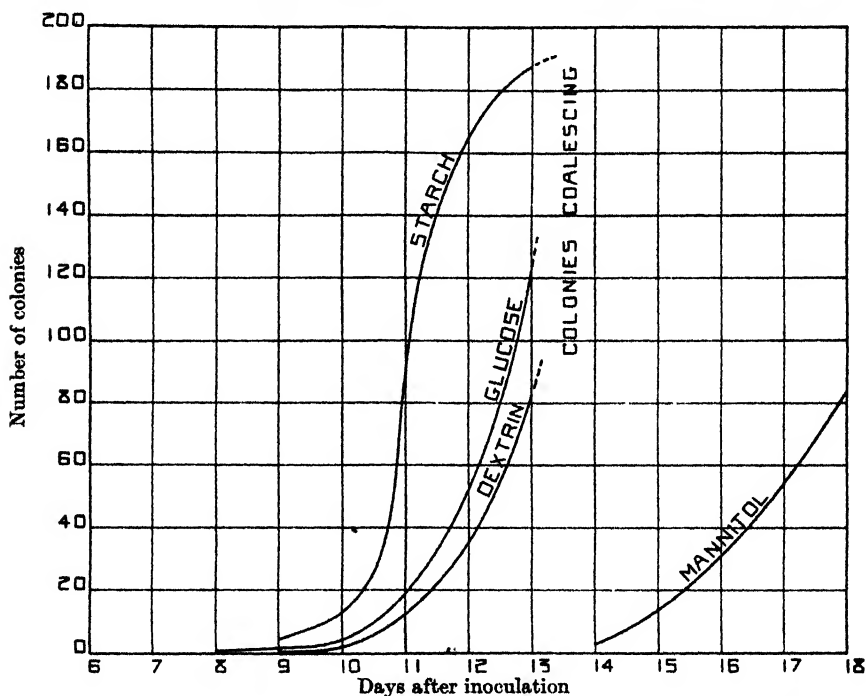


Fig. 1. Influence of certain carbon compounds on the initiation of growth by *Azotobacter*.

the control plates were treated with 2N  $H_2SO_4$  and dried out at 60° C. The rate at which *Azotobacter* initiated growth on the various media was determined by a daily count of the colonies. The means of these duplicate counts were plotted, and the curves fitted to the points thus obtained are reproduced in Fig. 1. It will be seen that there was a lag of eight days before any growth was apparent. A colony then developed on one of the pair of plates containing the glucose medium; one day later colonies were appearing on the starch and dextrin plates, but did not appear on the plates containing the mannitol medium until fourteen

days after inoculation. Whilst counting was still possible, that is before coalescence had set in, the highest number of colonies were visible on the starch plates. The mean of the final count on this medium was 187, and the maximum 212, showing that the soil used in this experiment contained approximately 800 organisms per gram. *Azotobacter* failed to develop on the lactic acid and sodium lactate plates.

The growth on the plates was stopped eighteen days after inoculation, and a determination made of the amount of nitrogen fixed which gave the following results:

Medium	pH	Nitrogen fixed, mg. Mean
Mannitol	4.8	0.20
Glucose	4.8	0.72
Dextrin	5.0	0.34
Starch	4.8	0.44

Information of some interest was to be derived from this experiment. It showed that mannitol does not necessarily provide the most suitable source of energy for the growth of a local strain of *Azotobacter*, and indicated that better conditions for nitrogen fixation, and presumably for growth, are supplied by a medium containing glucose; but that for the purpose of estimating the number of organisms in the soil a starch medium is more suitable, for on this the colonies remain discrete for a longer period. Furthermore, it was found that although growth on the mannitol plates was not initiated until after a lag of fourteen days, yet the soil contained a number of *Azotobacter* per gram which compared favourably with the figures reported for temperate soils (2, p. 27), and, as this same soil had been used in several of the previous experiments, there is reason to suppose that failure to initiate growth in such cases was due to some cause other than a low concentration of the organisms in the inoculum.

#### SUMMARY AND CONCLUSION

These observations have shown that the strains of *Azotobacter* occurring in certain Malayan soils are unable to initiate growth on standard media, except after a lag phase of considerable duration. Evidence has been obtained which suggests that this inability is not necessarily an indication of a scarcity of the organisms in the soil used as an inoculum, but may be due to factors associated with the composition of the culture medium.

In particular the strains of *Azotobacter* developing from these soils are checked by the presence of calcium carbonate in the medium. They



will grow well in a medium in which calcium chloride replaces calcium carbonate, but equally well where the medium contains but traces of calcium.

These strains can tolerate a wide range of hydrogen-ion concentration extending on the acid side to pH 3.6. Their existence invalidates the use for these soils of all such microbiological tests of lime requirement as make use of *Azotobacter* as indicator.

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# INVESTIGATIONS ON THE ROOT NODULE BACTERIA OF LEGUMINOUS PLANTS

## XVII. CONTINUED INVESTIGATIONS ON THE EFFECT OF AIR CONTENT OF THE MEDIUM ON THE DEVELOPMENT AND FUNCTION OF THE NODULE

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(With One Text-figure)

SOME time ago we showed (Virtanen and v. Hausen, 1935) that the air content of the medium influences largely the development and function of the nodules. This question has now been further studied by us, using inoculated, but otherwise sterile, pea cultures with a varied air content of the medium. The analytical procedure employed has been described in the paper cited above.

### WATER CULTURES

The following experiment (Exp. I) was made in order to ascertain the effect of air, and particularly of oxygen, on the formation and function of the nodules. In all cultures the roots and nodules were entirely submerged throughout the experimental period. The experiment consisted of nine pea cultures:

Cultures 1 and 2. Inoculated, no aeration.

Cultures 3 and 4. Inoculated. A stream of nitrogen (washed with concentrated sulphuric acid) was passed through the medium during the entire period of growth.

Cultures 5 and 6. Inoculated. A stream of air (washed with conc.  $\text{H}_2\text{SO}_4$ ) was passed through the liquid during the entire period of growth.

Culture 7. Inoculated. Nitrogen was first bubbled through the liquid for 22 days, then air was led through for 28 days.

Culture 8. Not inoculated; the medium contained 0.8017 gm. of  $(\text{NH}_4)_2\text{SO}_4$  per litre. No aeration.

Culture 9. Control, not inoculated; N-free medium.

The arrangement of the experiment is illustrated schematically by Fig. 1.

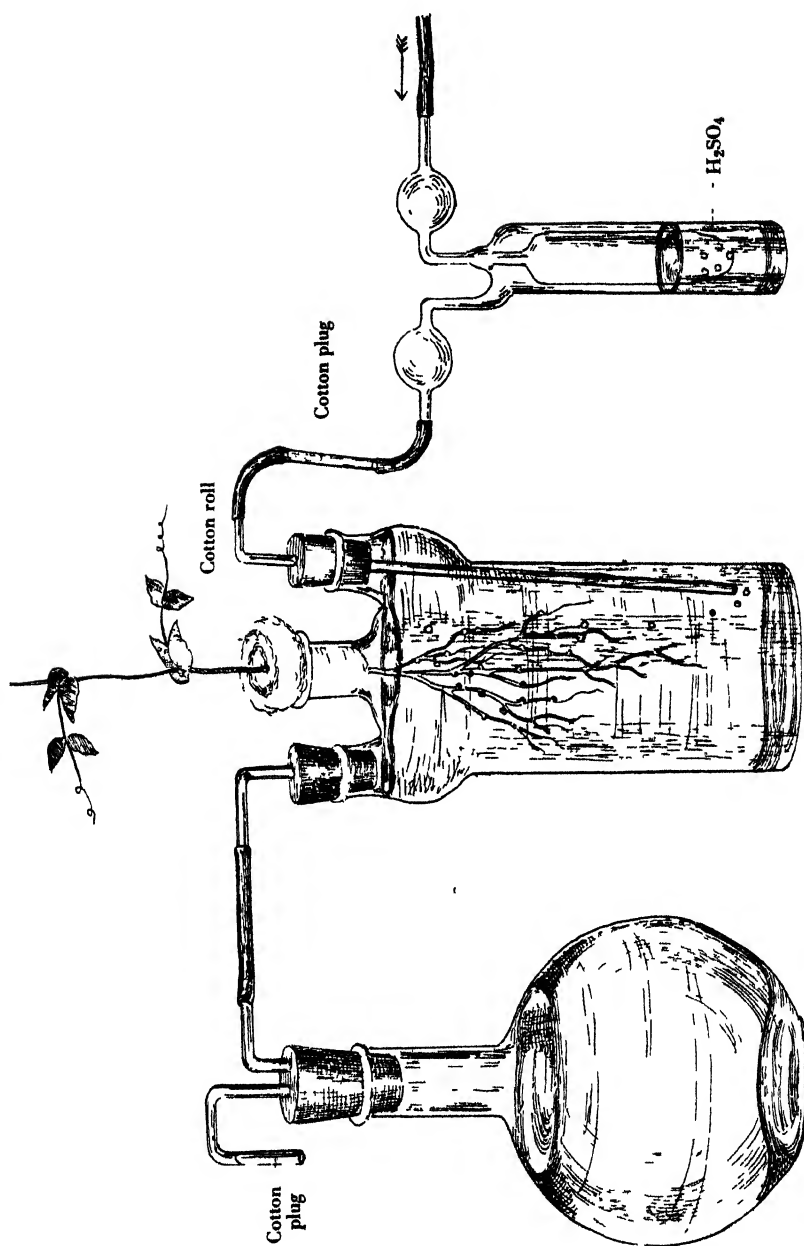


Fig. 1. Showing how the aeration of the cultures was arranged. The flask on the left served as reservoir from which sterile nutrient solution was added into the culture bottle to replace the solution which had evaporated.

Table I. *Water cultures of "Torstai" peas*

3-litre Woulff's bottles; 3 litres of N-free Hiltner's nutrient solution, pH 6.5; two sterile seedlings transferred Nov. 17, 1934. All plants harvested Jan. 4, 1935. The plants were grown in the greenhouse at 18-22° C. and illuminated with a 1000-watt lamp from a distance of 1 metre.

No. of culture	Treatment	Dry weight gm.	N mg.	Nodulation
1	Inoculated; no aeration	T.* 1.257 R. 0.303 M. —	19.04 6.44 1.26	Abundant; small nodules
2	"	T. 1.950 R. 0.603 M. —	38.92 9.80 1.26	"
3	Inoculated; N bubbled through the medium	T. 0.200 R. 0.227 M. —	3.36 6.58 2.94	None
4	"	T. 0.270 R. 0.250 M. —	3.23 6.30 1.96	"
5	Inoculated; air passed through the medium	T. 4.647 R. 1.110 M. —	123.76 24.36 1.54	Abundant; nodules were bigger than in Nos. 1-2
6	"	T. 4.108 R. 0.680 M. —	151.08 16.10 1.40	—
7	Inoculated; N passed through the liquid Nov. 17-Dec. 8; air Dec. 8-Jan. 4	T. 1.005 R. 0.275 M. —	13.16 5.60 1.26	First nodules appeared Dec. 16
8	Not inoculated; 0.8017 gm. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> per litre of medium	T. 1.680 R. 0.638 M. —	125.02 35.98 —	None
9	Control, not inoculated. No aeration	T. 0.493 R. 0.587 M. —	12.18 0	"

\* T. tops; R. roots; M. medium.

The results in Table I show that:

(1) No nodulation takes place when the oxygen in the medium is removed by passing a stream of sterile nitrogen gas through the liquid. The growth of the plants is consequently impossible.

(2) Abundant formation of well-developed nodules occurs when sterile air is bubbled through the medium during the entire period of growth; the plants grow profusely. Nitrogen is not excreted into the medium.

(3) As in (1), nodulation is prevented by passing nitrogen through the medium during the early stages of growth; however, when the nitrogen is, after 22 days, replaced by sterile air, nodulation occurs after a further 8 days, and the plants begin to grow.

(4) Abundant nodulation occurs even if no air is passed through the medium; the nodules, however, are much smaller than in the aerated cultures, and the plants grow very poorly. The nodulation is obviously made possible through the presence in the medium of small amounts of

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dissolved oxygen. The upper roots tend to rise to the surface of the liquid, and the nodules thus have access to air, whereupon a slight growth commences.

(5) The plants grow well on mineral nitrogen even if the cultures are not aerated. This shows that

(6) the pronounced indispensability of air to inoculated peas is ascribable to the air requirements of the nodules. Oxygen is indispensable for the formation and function of the nodules.

The above experiment was made in winter, under artificial illumination. Another similar experiment (Exp. II) was made in late autumn under natural light conditions, and consisted of three pea cultures:

- (1) Control, not inoculated.
- (2) Inoculated; no aeration.
- (3) Inoculated; air was passed through the medium during the entire period of growth.

In other respects the experimental conditions were similar to those in Exp. I. The roots and nodules were entirely submerged in the medium. Table II shows the results of this experiment.

Table II. *Water cultures of "Torstai" peas*

Experimental conditions as in Exp. I; natural illumination; two sterile seedlings transferred into each flask on Aug. 15; all harvested Oct. 3.

No. of culture	Treatment		Dry weight gm.	N mg.
1	Control, not inoculated: no aeration	T. R.	0.426 0.311	7.42 4.62
			0.737 } 12.04	
			Length of the plants 50 cm.	
2	Inoculated; no aeration	T. R.	1.441 0.431	29.12 9.38
			1.872 } 38.50	
			Length of the plants 78 cm.	
3	Inoculated; aerated	T. R.	3.796 0.516	62.72 14.98
			4.312 } 77.70	
			Length of the plants 110 cm.	

It will be seen that the results are closely analogous to those given in Table I.

### SAND CULTURES

We have paid particular attention to the effect of the air content of the medium on the function of the nodules in quartz sand cultures. A number of different experiments has been carried out with sterile pea cultures. In some cultures the sand was only just moistened with the nutrient solution, the moisture content of the medium being about 20 per cent. In these cultures the air content of the sand was thus fairly

high. In other cultures the sand was entirely submerged in the nutrient solution. Some of these cultures were aerated by passing a stream of sterile air through the sand medium. The aeration was facilitated by placing at the bottom of the flask a layer of pumice into which the air inlet tube extended. The treatment of the different cultures in this experiment (Exp. III) was as follows:

(1) Control, not inoculated; the sand was only just moistened with the nutrient solution; no aeration.

(2) Inoculated; otherwise as in (1).

(3) Inoculated; the sand was entirely submerged; no aeration.

(4) As in (3); aerated.

Each flask contained 1.4 kg. of quartz sand, the initial nitrogen content of which was 3.9 mg. This amount of N was subtracted from the final N values for the sand in the different cultures (except the control). In Table III the nitrogen values for the sand thus indicate the true increase in N, due to the excretion of nitrogenous compounds from the nodules. All nitrogen determinations on sand were made by the improved method described in the earlier paper (Virtanen and v. Hausen, 1935). It is clear from Table III that the peas grow poorly if the sand is entirely submerged in the medium. The growth is distinctly improved when a stream of air is bubbled through the sand. Aeration of the culture also increases the rate of excretion of nitrogenous compounds into the sand. Similarly it is seen that the peas grow well in sand which has been only just moistened with the nutrient solution and consequently has a fairly high air content. However, the best results were obtained by continuous aeration of the cultures, a fact which clearly proves the importance of an adequate air supply to the roots and nodules.

Table III. *Sand cultures of "Torstai" peas*

1-litre suction flasks; 1.4 kg. sand; two sterile seedlings transferred into each flask Jan. 12; harvested Feb. 18.

No. of culture	Treatment		Dry weight gm.	N mg.
1	Control, not inoculated. Sand moistened; no aeration	T.	0.314	12.74
		R.	0.088	2.52
		M.	—	3.9
2	Inoculated, otherwise as in No. 1	T.	2.380	52.08
		R.	0.457	11.20
		M.	—	33.60
3	Inoculated; sand en- tirely submerged; no aeration	T.	1.287	18.62
		R.	0.369	8.12
		M.	—	25.5
4	As in No. 3, but aerated	T.	3.207	65.80
		R.	0.426	12.88
		M.	—	40.80

## EXPERIMENT IV

Two 3-litre suction flasks were used as culture vessels in this experiment. One of the flasks was filled with sand (4.9 kg.) up to the neck. The sand was moistened with the nutrient solution. The other flask contained only 1.5 kg. sand. In this flask, only the tips of the roots were embedded in the sand, whereas the upper part of the roots was exposed to air. This was accomplished by keeping the flask filled with the nutrient solution until the roots had had time to reach the sand, after which the plants were allowed to transpire the major part of the liquid; fresh solution only being added to keep the sand just moist. No aeration was given in either case. The nitrogen values for the sand at the conclusion of the experiment have been corrected for the initial N-content of the sand.

Table IV. *Sand cultures of "Torstai" peas*

3-litre suction flasks, one filled with sand (4.9 kg.) up to the neck, the other containing only 1.5 kg. of sand at the bottom. Other experimental conditions as in Exp. III. No aeration. Two seedlings transferred into either flask Jan. 12; harvested Feb. 18.

No. of culture	Treatment		Dry weight gm.		N mg.
1	The roots were entirely embedded in the sand; inoculated	T.	2.010	} 2.502	52.64
		R.	0.492		11.06
		M.	—		75.20
2	Only the tips of the roots were embedded; other portions exposed to air; inoculated	T.	3.790	} 4.540	100.10
		R.	0.750		27.58
		M.	—		25.20

A powerful excretion of nitrogen has taken place in culture No. 1, where the roots were entirely embedded in the sand. On the other hand, in culture No. 2, where the major part of the roots was exposed to air, the excretion has been slight, whereas the plants have grown much more vigorously than in No. 1.

The above results confirm our earlier view that the roots and nodules must be in direct contact with solid materials in order that an excretion of nitrogen may take place. There is also some indication that the growth of the plant is improved when the N excretion is artificially prevented by allowing only the root tips to grow in sand, the major part of the root system, with the nodules, being exposed to air. A powerful excretion of nitrogen would thus appear to be harmful to the host plant in depriving it of its nitrogen supply. However, as in culture No. 2 the access of air to the roots was better than in No. 1, it is not possible to draw definite conclusions on this point. In associated cultures of legumes and non-legumes it often appears that the non-legumes deprive the legumes of

their nitrogenous food, thus impairing their growth. This is particularly the case when the number of the non-legumes is much greater than that of the legumes.

#### SUMMARY

The effect of the air content of the medium on the development and function of the nodule was studied with sterile cultures of peas inoculated with their appropriate strain of nodule bacteria.

In water cultures the plant grows well without added nitrogen, even though all nodules are submerged, if air is bubbled through the medium during the period of growth. Abundant nodulation occurs also in non-aerated liquid cultures; the nodules, however, are much smaller than in aerated cultures, and the plant grows poorly. Subsequent aeration of such cultures results in a distinct increase in the size of the nodules and improvement in the growth of the plant.

Nodulation is completely prevented by passing a stream of gaseous N through the medium. That oxygen is indispensable for the function of the nodules is shown also by the fact that the peas grow excellently on added nitrogen in non-aerated cultures.

Nitrogenous compounds are not excreted into the medium in inoculated water cultures of peas, even when the cultures are aerated and the plant grows profusely.

In sand cultures where the sand was entirely submerged in the nutrient solution, a profuse growth took place when air was passed through the sand. Under these conditions considerable amounts of nitrogen were excreted into the medium. It is apparent therefore that the roots and nodules must be in direct contact with solid materials in order that nitrogen may be excreted. This view is further supported by the fact that in cultures where only the root tips were embedded in the sand, the upper part of the roots being exposed to air, the plant grew excellently whereas the rate of excretion was much lower than when the roots were entirely embedded in the sand.

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# STUDIES ON THE WINTER HARDINESS OF CLOVER

## I. EFFECT OF CUTTING ON THE CARBOHYDRATE RESERVES IN RED CLOVER ROOTS

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(With One Text-figure)

AN important feature in modern dairy farming is the tendency to lower the cost of milk production and to obviate as far as possible the necessity for using purchased concentrates in cattle feeding. Increasing attention is therefore being paid to the cultivation of leguminous crops. In this respect the perennial legumes offer great possibilities, owing to their low cost of cultivation. In northern countries like Finland, red clover ranks first among the perennial legumes, and consequently any new light on the cultural characteristics of this plant would be of value as a contribution towards development in dairy farming.

The introduction of the A.I.V. process has brought about an essential change in Finnish farming practice. Whereas previously the clover fields were cut only once a season, it has now become customary to cut the fields two and even three times each year. This change has naturally created new problems, particularly as regards the effect of frequent cutting on the wintering of clover. Field trials and practical farming have failed to show clearly whether the more frequent cutting actually affects the winter hardiness of clover. Our farmers sometimes claim that cutting in autumn results in a thinning of the clover stand in the following season. However, since this claim is based merely on practical observations of doubtful validity, and not on controlled experiments with uncut parallel plots, the question must be regarded as more or less unsettled. It is possible, of course, that there may be certain periods in autumn, during which it is not advisable to cut the clover fields, although the evidence to support this assumption is not convincing.

It is generally held that alfalfa should not be cut in late autumn. According to Sylvén (1934) it has a long critical cutting period in autumn,

whereas a very late cutting does not endanger the wintering. Field trials and practical experience in this country show that, if there is any critical period for cutting clover in autumn, this period is much shorter than with alfalfa.

The wintering of plants is obviously dependent on the content of reserve materials in the underground organs. As far as we know, no work has been done regarding the effect of different cutting practices on the composition of red clover roots. The experiments recorded here form a preliminary part of our investigations on this subject. We have paid particular attention to the carbohydrate constituents of the roots, since these compounds are readily utilised by the plant and thus form the most important reserve materials at the time when growth commences in spring, often under very difficult climatic conditions. When our experimental work was finished we found that Grandfield (1935) had recently published results of corresponding work with alfalfa roots. He showed that after each cutting, the carbohydrate content reached a minimum in about 20 days, and rose again to its original level in a further 10–15 days. The low carbohydrate level period after each cutting was thus about 4–5 weeks, and did not vary significantly with the season. Early and frequent cutting resulted in a lower, and infrequent cutting in a higher carbohydrate content of the roots when winter arrived. Grandfield's findings thus corroborate Sylvén's view that there is a long critical period for cutting alfalfa in autumn.

#### EXPERIMENTAL

The root material was obtained from a second year's field of red clover in the vicinity of Helsinki. Prior to the experiment root samples were taken from several parts of the field, and an area was ultimately chosen in which the material proved to be very homogeneous throughout. This area was then divided into five plots, of which one (plot 1) was left uncut throughout the season whilst the others were cut at different stages of growth. Plots 2, 3, 4 and 5 were all cut on June 26, just before flowering. Plot 2 was then left uncut until the end of the season, whilst a second cut was made on the remaining plots in late summer. Plot 3 was cut for the second time on August 26, in full bloom. The second cutting on plot 4 was made on September 7, at a late flowering stage, and on plot 5 on October 4, when the plants were fully mature and seeding.

For the determination of the carbohydrate reserves, about twenty roots were picked at random from different parts of the plot. The green

portions were removed and the roots washed with cold water and dried with towel and filter paper. A composite sample was then prepared by cutting the roots into pieces of about 0.5 c.c. and thoroughly mixing

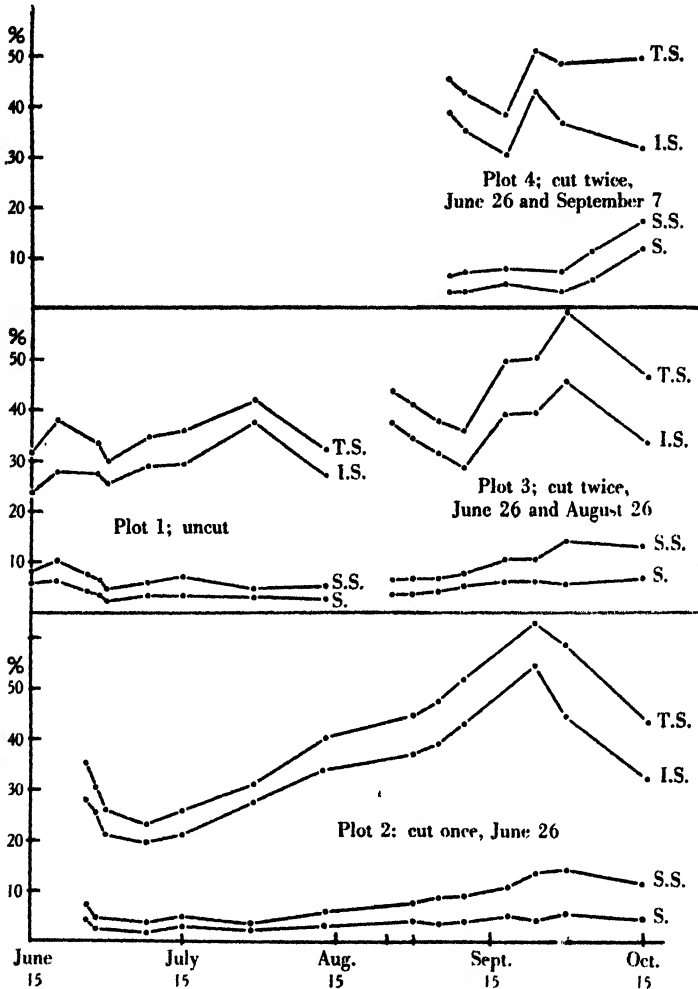


Fig. 1.

the lot. About 25-30 gm. of the material was weighed and thrown into 100 ml. of boiling 95 per cent. alcohol. After boiling for 1 hour the alcohol was filtered off through asbestos and the residue was further extracted three times with 100 ml. 80 per cent. alcohol, each time for

30 min. The combined alcoholic extracts were evaporated at 40° under reduced pressure. The residue was transferred into a 100-ml. volumetric flask and made up to the mark with water. After clearing with neutral lead acetate and deleading with disodium phosphate, sugar determinations were made on aliquots of the extract by the method of Lehmann-Maquenne-Schoorl. Reducing sugars were first determined directly from the extract. For the determination of the total soluble sugars, an aliquot of the extract was hydrolysed with 3 per cent. HCl on the water-bath for 30 min., neutralised and made up to a suitable volume. Sucrose was estimated from the increase in the reducing power of the extract, following a hydrolysis with 1 per cent. HCl at 70° for 20 min.

The alcohol-insoluble residue was dried at 102–104° for 12 hours and weighed. The dry weight so obtained was taken as basis for calculating the percentage values of the different sugar fractions. All values are thus given in percentages of the dry matter which remained undissolved after the alcoholic extraction. In our opinion, this basis of measurement ("extracted dry weight" basis) is more constant during the season than the dry weight basis usually employed. The extracted dry material was hydrolysed on the water-bath with 3 per cent. HCl for 3 hours, neutralised approximately with 20 per cent. NaOH, filtered and made up to 1000 ml. with water. Aliquots of this solution were then taken for the estimation of the total insoluble sugars. The determinations were made according to Bertrand's method and the results were calculated as glucose. Table I illustrates the effect of cutting on the total and insoluble sugars in clover roots, while the corresponding changes in sucrose and other soluble sugars are given in Table II. The experimental results are illustrated graphically in Fig. 1.

#### DISCUSSION

*Plot 1; uncut.* As the first samplings were made towards the middle of June, no experimental data are available concerning the changes in the carbohydrate reserves during the spring and early summer. It is known that the carbohydrate content of the roots decreases during the development of the assimilating organs, and rises again when these have been formed and the products of assimilation begin to be deposited in the roots. In the present experiment this phase takes place during early June. The first maximum was reached on June 20. A continuous fall then occurred, particularly in the soluble sugars, until July 1. This fall coincided with a spell of very hot weather and is thus probably not characteristic of the normal season. A second maximum was reached

Date of sampling	Plot 1			Plot 2			Plot 3			Plot 4			Plot 5		
	Cut	Total sugars	In- soluble sugars	Cut	Total sugars	In- soluble sugars	Cut	Total sugars	In- soluble sugars	Cut	Total sugars	In- soluble sugars	Cut	Total sugars	In- soluble sugars
June 15	Uncut	31.6	23.4				June 26	—	—	June 26	—	—	June 26	—	—
20	—	37.8	27.7												
26	—	34.5	27.2												
28	—	33.3	27.0												
July 1	—	29.9	25.4												
8	—	34.7	29.0												
15	—	35.9	29.1												
29	—	42.0	37.5												
Aug. 13	—	32.2	27.1				Aug. 26	43.7	37.4						
26	—	—	—				—	41.2	34.6						
30	—	—	—				—	38.2	31.6						
Sept. 5	—	—	—				—	—	—						
7	—	—	—				—	36.1	28.4	Sept. 7	45.5	38.8	—	—	—
10	—	—	—				—	49.8	39.3	—	42.9	36.6	—	—	—
18	—	—	—				—	53.7	42.8	—	38.5	30.5	—	—	—
24	—	—	—				—	62.9	49.4	—	50.8	43.2	—	—	—
30	—	—	—				—	58.7	44.6	—	48.3	36.8	—	—	—
Oct. 4	—	—	—				—	—	—	Oct. 4	—	—	—	—	—
10	—	—	—				—	43.3	31.8	—	49.3	31.8	—	50.7	37.3

Table II. *Effect of cutting on the content of sucrose and the total soluble sugars in red clover roots*

Date of sampling	Plot 1			Plot 2			Plot 3			Plot 4			Plot 5		
	Cut	Soluble sugars	Su- crose	Cut	Soluble sugars	Su- crose	Cut	Soluble sugars	Su- crose	Cut	Soluble sugars	Su- crose	Cut	Soluble sugars	Su- crose
June 15	Uncut	8.2	5.6				June 26	—	—	June 26	—	—	June 26	—	—
20	—	10.1	6.1												
26	—	7.3	4.0												
28	—	6.3	2.3												
July 1	—	4.5	2.0												
8	—	5.7	3.2												
15	—	6.8	3.2												
29	—	4.5	2.7												
Aug. 13	—	5.1	2.4				Aug. 26	6.3	3.3						
26	—	—	—				—	6.6	3.3						
30	—	—	—				—	6.6	3.8						
Sept. 5	—	—	—				—	—	—	Sept. 7	6.7	3.5	—	—	—
7	—	—	—				—	—	—	—	7.3	3.6	—	—	—
10	—	—	—				—	—	—	—	8.0	4.9	—	—	—
18	—	—	—				—	10.5	6.9	—	10.5	6.8	—	—	—
24	—	—	—				—	10.6	6.8	—	7.5	3.7	—	—	—
30	—	—	—				—	13.9	6.4	—	11.5	5.9	—	—	—
Oct. 4	—	—	—				—	—	—	—	—	—	—	—	—
16	—	—	—				—	12.9	6.6	—	17.5	12.1	—	13.4	6.2

towards the end of July, when the plants were already long past the flowering stage. The content of insoluble sugars in the roots was then 37.5 per cent., against 27.7 per cent. on June 20. The last phase is characterised by a rapid fall in the insoluble carbohydrates, owing to the fact that the prostrate clover plants were almost completely overshadowed by the taller grasses. Under these conditions, the rate of assimilation by the over-mature clover is naturally very low, and as respiration and other sugar-consuming processes are not retarded, they cause a large decrease in the insoluble carbohydrates.

The changes in the soluble sugars were very small throughout the experimental period.

*Plot 2; cut once June 26.* Cutting of the field causes an abrupt fall in the sugar content of the roots. Within 5 days the insoluble sugars decreased from 28.0 to 21.2 per cent., and the soluble sugars from 7.2 to 4.9 per cent. The lowest values are obtained about 2 weeks after cutting, when the total sugar content of the roots is only 23.2 per cent., against 34.7 per cent. in the corresponding sample from the uncut field. Then follows a continuous increase, slow at first, then fairly rapid, until the total sugar value reaches a maximum of 62.9 per cent. on September 24. This increase is chiefly due to the increase in the insoluble sugars—from 19.4 to 49.4 per cent.—while the soluble sugars increase from 3.8 to 13.5 per cent. The decreasing rate of assimilation in late summer and autumn then causes a steep decline, chiefly in the insoluble sugars, the final concentration of which, in the middle of October, is 31.8 per cent. At this stage there is also a considerable proportion (11.5 per cent.) of soluble sugars in the roots, so that the total carbohydrate reserves amount to 43.3 per cent., against 32.2 per cent. on the uncut field towards the middle of August.

*Plot 3; cut twice, June 26 and August 26.* At the time of the second cutting the values for the different sugar fractions are similar to the corresponding values from plot 2. Cutting again results in a rapid decrease of the insoluble sugars, whereas the soluble sugars are not affected. Even in this case the minimum is reached in about 2 weeks, but the refilling of the root reserves is accomplished much more quickly than in the corresponding experiment in early summer. In the latter case the original carbohydrate level was reached in about 6 weeks after the cutting, whereas in plot 3 this occurred after 3 weeks. The total sugar values then rose to a maximum of 59.6 per cent. on September 30, and fell again during the first weeks of October to 46.6 per cent. The changes in the soluble sugars are analogous to the corresponding changes in

plot 2. The final carbohydrate content of the roots from plot 3 is slightly higher than when no second cut was made.

*Plot 4; cut twice, June 26 and September 4.* The effect of the second cutting is similar to that observed in plot 3, although the recovery is here still more rapid, the pre-cutting level being reached after about 2 weeks. Owing to the advanced season, the maximum values were considerably lower than those obtained with earlier cuttings. It is interesting to note, however, that there is no rapid decline in the total sugars once the maximum is reached. The increase in soluble sugars almost completely compensates for the decrease in insoluble carbohydrates. Consequently, the total carbohydrate content of the roots at the conclusion of the experiment is considerably higher than in plots 2 and 3.

*Plot 5; cut twice, June 26 and October 4.* This experiment was made to ascertain the effect of a very late second cutting on the wintering of clover. In the sample taken on October 16, the total carbohydrate value slightly exceeded that in plot 4, while the content of insoluble sugars was distinctly higher than in any of the plots from which the second cut was made earlier in the season.

The above results show that the total carbohydrate reserves in clover roots are the larger, the later the second cut. They also show that the loss in root sugars, caused by cutting, is made up much more rapidly in late summer than in June-July. This fact is of considerable importance, for it is generally assumed that a high sugar content in the roots substantially contributes to the successful wintering of the plant. It is therefore reasonable to assume that there is a critical period after a late cutting, when the carbohydrate reserves in the roots are exhausted, owing to the building up of the assimilating organs. If this critical period coincides with the onset of frosts, the wintering of the clover is greatly endangered. Fortunately, however, the critical period seems to be much shorter in autumn than in summer. Further work is now in progress to determine the percentage survival of plants on the differently treated plots and also to study the effect of frequent cutting on the carbohydrate root reserves in a first year's clover field.

#### SUMMARY

The effect of different cutting practices on the carbohydrate root reserves of Finnish red clover was studied. Cutting causes a rapid decrease in the insoluble sugars. The minimum is reached in about 12-15 days, independently of the time of cutting. However, the initial carbo-

hydrate level is reached much more quickly in autumn than in June-July. The carbohydrate content of the roots in autumn is higher the later the second cut.

Cutting does not significantly affect the content of sucrose and other soluble sugars, the values for which are very low during June-August, but rise towards autumn.

The expenses of this investigation have been met by a grant from Messrs Valio Co-operative Butter Export Association, to whom our sincerest thanks are due. We also wish to thank Dr H. Karström for much valuable help in sampling.

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# THE EFFECTS OF DIFFERENT METHODS OF CASTRATION AND DOCKING ON THE GROWTH OF LAMBS

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DURING the last two years studies have been made on the rate of growth of lambs produced by the Cambridge University Farm flock. This flock comprises nearly 300 half-bred ewes (Border Leicester ram  $\times$  Cheviot ewe) bred in Scotland and purchased in the spring at the approximate age of 12 months.

Although cross-breds cannot provide genetically homogeneous material it is well known among practical men that these half-breds produce very uniform lambs. The ewes have generally been mated with Suffolk rams, but for the 1935 lamb crop three of the Suffolk rams were displaced by one Ryeland and two Southdowns. Since rams of three different breeds have been used there is a temptation to draw conclusions as to the relative merits of the progeny of the breeds; this would clearly be unjustifiable because one or two sires can never provide a fair representation for a breed. In the tables given in this paper the progeny from sires of different breeds are shown separately, not with the idea of comparing breeds, but in order to show the experimental comparison for each breed.

The following factors were studied:

- A. Castration—knife *v.* Burdizzo.
- B. Docking—hot iron *v.* Burdizzo and knife.
- C. Sex.

In the first two of these studies the methods now widely used in crop experimentation were adopted. The first step was to weigh each lamb; this was done 2–3 weeks after birth. The maximum age variation in any one group of lambs was only a few days and so they were paired to form “blocks” solely on the basis of this initial weight. The treatments were separately randomized over the members of each block and thus the modern statistical “layout” was obtained. In 1934 studies A and B were combined in the case of the ram lambs, blocks of four individuals being formed; since there was no interaction between methods

of castration and methods of docking the results are shown separately in Tables I and II. The treatments were carried out within 24 hours of the initial weighing and each lamb was weighed again 2 months later. Since the "layout" ensured equalisation at the outset, the conclusions are based on the final weights (at the approximate age of 10 weeks).

As it is impossible to randomize sex the above method could not be employed for study C. In this case therefore both initial weights and live weight gains are given (Tables III and IV).

#### A. METHODS OF CASTRATION

The generally adopted method of castration is to cut off the bottom of the scrotum, make a slit in the peritoneal covering of each testis, and withdraw the testes and the "cords". Whilst this method is certainly effective in de-sexing the animal, it is open to objection on account of the risk of infection; this latter is of special importance under warm climatic conditions.

The Burdizzo has been in use for some time, and is fairly well known. It is essentially a pair of blunt pincers designed to give considerable pressure and it is used to crush each "cord" separately. The common practice with calves is to crush each "cord" at two different places, but with lambs each "cord" is usually crushed only once. The Burdizzo method has the great advantage that it leaves no wound. It is rather slower in operation than the older method but its main disadvantage lies in an element of uncertainty. If a "cord" is completely missed the fact is usually advertised by the subsequent behaviour of the lamb, when the operation can be repeated, but occasionally the "cord" is incompletely crushed, in which case some testicular tissue survives, and some slight development of secondary sexual characters may occur. There is a considerable prejudice among butchers against this method on the score that the lambs so treated have thicker necks, and that the flesh is redder, more highly flavoured and tougher; this objection does not arise, of course, in the case of lambs slaughtered at an early age. It is very probable that the genesis of the butchers' prejudice is to be sought in the cases where incomplete crushing occurs, because when the operation is successfully performed no secondary sexual characters develop.

The results for this study are shown in Table I, where it will be seen that no difference approaches significance, although the experimental layout led to very low errors. Thus it may be concluded that there is no differential effect upon growth between the two methods of castration.

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Neither was there any evidence of differential effect on mortality; the total number of lambs included in the study was 158 and the total number of deaths was three, of which two had been castrated with the Burdizzo and one with the knife. When one member of a pair died the "block" was discarded.

Table I. *Castration. Mean final wt. (lb.)*

Breed of ram crossed with half-bred ewes	No. of pairs	Method of castration		S.E. of difference	<i>t</i>	Significance
		Knife	Burdizzo			
Suffolk (1934)	20	62.75	63.87	1.19	0.93	Insignificant
Suffolk (1935)	28	66.68	66.56	0.93	0.13	Insignificant
Ryeland (1935)	18	64.37	64.11	1.15	0.23	Insignificant
Southdown (1935)	10	57.35	58.12	1.31	0.59	Insignificant
Combined results	76	63.88	64.16	0.57	0.49	Insignificant

### B. METHODS OF DOCKING

A larger Burdizzo (constructed for castrating calves) may be used for docking lambs, the object being to reduce bleeding. The method is to crush the tail and then, while the Burdizzo is still clenched, to cut off just below the point of crushing. Whilst easy in operation this method is probably cruel; the rate of mortality was 2 out of 46. Where crushing is complete, mortification sets in below the point of application and the remaining piece of tail eventually falls off; this may provide a second opportunity for infection.

The method of docking usually adopted on the University Farm is to sear off the tail with a hot iron; this effectively prevents infection, provided care is exercised not to knock off the scab whilst releasing the animal.

The two methods described were compared in 1934 with the results shown in Table II; the comparison was not continued in the following year because the Burdizzo method appeared to have little to recommend it and was thought to be cruel.

Table II. *Docking. Suffolk crosses, 1934. Mean final wt. (lb.)*

Sex of lambs	No. of pairs	Method of docking		S.E. of difference	<i>t</i>	Significance
		Hot iron	Burdizzo			
*Rams	20	63.91	62.73	1.19	0.99	Insignificant
Ewes	24	60.03	59.54	1.80	0.27	Insignificant
Rams and ewes	44	61.79	60.99	1.08	0.74	Insignificant

\* Throughout this paper male lambs are referred to as rams irrespective of whether they have been castrated or not.

The table shows no difference in final weight between these two methods. It is fully realised that the Burdizzo method may be preferable to that of merely cutting off the tail; the latter is practised on many farms, but it is open to objection in that considerable bleeding may ensue, and that no protection from infection is given.

### C. SEX

In the above two studies information as to the relative weights of ram and ewe lambs was obtained, and, to continue this comparison, ewe lambs were weighed in 1935 although they were subjected to no experimental treatment. The initial and final weights for the two sexes are given in Tables III and IV.

Table III. *Sex. Mean initial wt. (lb.)*

Breed of ram crossed with half-bred ewes	Rams		Ewes		S.E. of difference	<i>t</i>	Significance
	No.	Mean	No.	Mean			
Suffolk (1934)	40	19.99	48	18.72	0.58	2.19	Rams > ewes ( $P < 0.02$ )
Suffolk (1935)	56	27.71	68	27.57	0.89	0.16	Insignificant
Ryeland (1935)	36	23.57	27	22.36	1.07	1.13	Insignificant
Southdown (1935)	20	20.67	23	19.05	1.47	1.10	Insignificant
Combined results	152	23.77	166	22.98	0.48	1.65	Insignificant

Table IV. *Sex. Mean live weight gains (lb.)*

Breed of ram crossed with half-bred ewes	Rams		Ewes		S.E. of difference	<i>t</i>	Significance
	No.	Mean	No.	Mean			
Suffolk (1934)	40	43.32	48	41.07	1.13	1.99	Rams > ewes ( $P < 0.05$ )
Suffolk (1935)	56	38.92	68	38.61	0.75	0.41	Insignificant
Ryeland (1935)	36	40.67	27	38.31	0.97	2.43	Rams > ewes ( $P < 0.02$ )
Southdown (1935)	20	37.06	23	34.93	1.31	1.63	Insignificant
Combined results	152	40.25	166	38.77	0.50	2.96	Rams > ewes ( $P < 0.01$ )

It will be observed that, as regards initial weight, in 1934 the rams were higher, but that no other significant difference was found in any of the groups, nor with both years' results together. As regards live weight gain in 2 months, there was a significant difference in favour of ram lambs in two of the groups and also when all were combined. The relative weights of the sexes have been discussed at length by Hammond (1) who showed that male lambs made greater live weight gains, except in the case of triplets with which the shock of castration appeared to inflict a more serious check. The lambs used in these studies were not grouped into singles, twins and triplets, but twins greatly predominated.

## 300 *Different Methods of Castration and Docking of Lambs*

### SUMMARY

1. Two methods of castrating male lambs have been compared—the Burdizzo and knife methods. No difference was found in subsequent growth rate.

2. Two methods of docking male and female lambs have also been compared—searing and crushing with the Burdizzo. No difference was found in subsequent growth rate.

3. There was a tendency for ram lambs to have a higher initial weight than ewe lambs, and their rate of growth between the ages of 2 and 10 weeks was definitely greater than that of ewe lambs.

### REFERENCE

- (1) HAMMOND, J. *Growth and Development of Mutton Qualities in the Sheep* (1932), pp. 13-27. Edinburgh: Oliver and Boyd.

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# INCOMPLETE LATIN SQUARES

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(With Two Text-figures)

## INTRODUCTION

IN experiments arranged in the form of a Latin square, it sometimes happens that all the values belonging to one row or one column are defective or non-existent. Again, all the values belonging to one treatment may be missing, either owing to the failure of that treatment, or the fact that the treatment is such that no final values can result in the phase of the experiment under consideration; as, for example, has recently occurred in a green manuring experiment on maize, where in certain years some of the plots were growing green manure and the remainder maize.

In such cases the ordinary procedure of the analysis of variance breaks down, owing to non-orthogonality. In the case of a missing treatment the rows and columns are not orthogonal, since each row has a value from one column missing, and *vice versa*. The simplest procedure is to treat the experiment as if it were a randomized block arrangement, and eliminate either rows or columns. Such a procedure is not valid, however, since additional restrictions have been imposed on the arrangement beyond those required for randomized blocks. If both rows and columns produce large effects serious over-estimation of the errors affecting the treatment comparisons will result.

A valid estimate of the experimental error can be made with little extra labour when a single row, column or treatment is missing, or when both a row and a column, or either, and a treatment are missing. In view of the great utility and wide application of Latin square arrangements both in agricultural field trials and in other fields of biological experimentation it is worth placing the procedure on record.

This procedure, moreover, has a wider interest than that of making the best of defective experiments, for, as will be shown, incomplete Latin squares of the above types are perfectly valid experimental arrangements, in that all bias is eliminated from the estimate of error by the

accepted processes of randomization. It is therefore quite legitimate to lay down a Latin square with, for example, a row missing. Though such a practice is not to be recommended in agricultural field trials, where there is rarely any definite limit to the number of plots, it may be very valuable in other types of experimentation where the available experimental material is such that it is not possible to form a complete square. In a pig experiment, for instance, a contemplated  $6 \times 6$  Latin square involving treatments, pens and litters might be rendered impossible by the fact that some of the litters contained only five suitable pigs; in such a case a Latin square arrangement with one pen missing could be employed, thus enabling litter effects to be eliminated.

It is worth noting that similar arrangements in incomplete randomized blocks are also possible, and that with these there is no restriction as to the number of experimental units per block, which may have any constant value less than the number of treatments, though for some block sizes a large number of replications is required to give the necessary symmetry. It is intended to publish an account of such arrangements elsewhere.

### THEORY

The standard method of dealing with one or more missing values (3) breaks down when all the values of a given treatment, row, or column are missing. The problem can best be approached by the method of fitting constants (2).

Table I

Row	Column						Total
	1	2	3	.....	$p$		
1	(1)*	(2)*	(3)*	.....	( $p$ )*		$R_1$
2	$y_{21p}$	$y_{221}$	$y_{232}$	.....	$y_{2p(p-1)}$		$R_2$
3	$y_{31(p-1)}$	$y_{32p}$	$y_{331}$	.....	$y_{3p(p-2)}$		$R_3$
.....	.....	.....	.....	.....	.....		.....
$p$	$y_{p12}$	$y_{p23}$	$y_{p34}$	.....	$y_{pp1}$		$R_p$
Total	$C_1$	$C_2$	$C_3$	.....	$C_p$		$G$

Treatment totals:  $T_1 = y_{221} + y_{322} + \dots + y_{pp1}$ ,

$T_2 = y_{232} + y_{342} + \dots + y_{p12}$ , etc.

\* Missing. The number in the bracket indicates the treatment that is required to complete the Latin square.

We will first consider the case where the whole of one row of a  $p \times p$  Latin square is missing. Let the remaining values of the experiment be given by  $y_{uvw}$ , where the suffix  $u$  denotes the row, and has all values from 2 to  $p$ , the suffix  $v$  denotes the column, and has all values from 1 to  $p$ , and the suffix  $w$  denotes the treatment and has some value from 1 to  $p$  given by the Latin square arrangement in conjunction with

each pair of values of  $u$  and  $v$ . The treatment that is missing from column 1 may be numbered 1, etc., without loss of generality. Let the row, column and treatment totals be denoted by  $R_u$ ,  $C_v$ ,  $T_w$  respectively, and the grand total by  $G$ . Take the treatment arrangement given by Table I.

Let the constants be as follows:

$$\begin{array}{llll} \text{Mean:} & m. & & \\ \text{Rows:} & r_2, r_3, \dots, r_p & S(r) = 0. & \\ \text{Columns:} & c_1, c_2, c_3, \dots, c_p & S(c) = 0. & \\ \text{Treatments:} & t_1, t_2, t_3, \dots, t_p & S(t) = 0. & \end{array}$$

The equations for the residuals  $\epsilon_{uv}$  are

$$y_{21p} = m + r_2 + c_1 + t_p + \epsilon_{21}$$

$$y_{221} = m + r_2 + c_2 + t_1 + \epsilon_{22}$$

$$y_{31p-1} = m + r_3 + c_1 + t_{p-1} + \epsilon_{31}$$

$$\dots\dots\dots$$

etc.

By the ordinary method of least squares the values of  $m$ ,  $r$ ,  $c$ ,  $t$  which make the sum of the squares of all the  $\epsilon$ 's a minimum are

$$\begin{array}{ll} p(p-1)m & = G \\ pm + pr_2 & = R_2 \end{array}$$

$$\begin{array}{l} (p-1)m + (p-1)c_1 - t_1 = C_1 \\ (p-1)m + (p-1)c_2 - t_2 = C_2 \end{array}$$

$$\dots\dots\dots$$

$$\begin{array}{l} (p-1)m - c_1 + (p-1)t_1 = T_1 \\ (p-1)m - c_2 + (p-1)t_2 = T_2 \end{array}$$

$$\dots\dots\dots$$

The constant  $m$  is therefore the ordinary arithmetic mean, and the row effects, represented by the  $r$ 's, are orthogonal with the column and treatment effects.

The equations for the  $c$ 's and  $t$ 's fall into pairs, the first containing  $c_1$  and  $t_1$ , the second  $c_2$  and  $t_2$ , etc. Combining the first pair, we have

$$\begin{array}{l} p(p-1)m + p(p-2)c_1 = (p-1)C_1 + T_1, \\ p(p-1)m + p(p-2)t_1 = (p-1)T_1 + C_1, \end{array}$$

with similar equations for the other pairs.

The reduction in the sum of squares due to fitting both the  $c$ 's and  $t$ 's is

$$c_1C_1 + c_2C_2 + \dots + t_1T_1 + t_2T_2 + \dots$$



On substituting for the  $c$ 's and  $t$ 's this reduces to

$$\frac{1}{p(p-2)} [(p-1) \{ (C_1^2 + C_2^2 + \dots) + (T_1^2 + T_2^2 + \dots) \} + 2 (C_1 T_1 + C_2 T_2 + \dots) - 2G^2],$$

or the sum of

$$\frac{1}{(p-1)} (C_1^2 + C_2^2 + \dots) - \frac{G^2}{p(p-1)}$$

and

$$\frac{1}{p(p-1)(p-2)} [(p-1) T_1 + C_1]^2 + [(p-1) T_2 + C_2]^2 + \dots - \frac{G^2}{(p-1)(p-2)}.$$

The first of the last two expressions represents the sum of squares due to columns when treatments are ignored. The second represents the sum of squares due to treatments after allowing for column effects, and therefore gives the appropriate sum of squares with which to test treatments ( $p-1$  degrees of freedom). The altered correction for the mean in the last expression should be noted. The whole expression is the sum of the squares of the deviations of the quantities  $(p-1)T + C$  from their mean  $G$ , divided by  $p(p-1)(p-2)$ .

The effects of the treatments, in units of a single value or "plot yield", and freed from column effects, are given by the differences between the quantities

$$\frac{(p-1)T_1 + C_1}{p(p-2)}, \quad \frac{(p-1)T_2 + C_2}{p(p-2)}, \dots$$

The standard error of each difference is

$$\sqrt{2} \times \sqrt{\frac{(p-1)}{p(p-2)}} \times \text{the standard error of a single value.}$$

If one treatment is missing instead of a row or column the procedure is the same, using row instead of treatment totals. The sum of squares due to rows after eliminating columns is the sum of the squares of the deviations of  $(p-1)R + C$  divided by  $p(p-1)(p-2)$ , each  $R$  being associated with that  $C$  which would have included the same missing value.

It is worth noting that the reduction in the error sum of squares due to the omission of one treatment is directly calculable, being the sum of the squares of the deviations of the  $p$  quantities

$$R + C - (p-2)y \text{ or } R' + C' - py,$$

divided by  $p(p-2)$ , where  $y$  is the yield of the treatment on any one plot, and the  $R$  and  $C$  are the totals of the corresponding row and column, dashes indicating the inclusion of the omitted treatment. This sum of squares corresponds to  $p-1$  degrees of freedom and can be

compared with the remaining  $(p-1)(p-3)$  error degrees of freedom to test whether the treatment in question is showing differential effects on the different plots of the experiment.

If more than one row is missing the equations for the  $c$ 's and  $t$ 's no longer fall into pairs, since each column has more than one missing treatment. The simplicity of the above solution is consequently lost.

If one row and one column are missing a neat solution is still available. One treatment, that which is common to the missing row and column, is differentiated from the others by having only one missing value. If this is treatment 1 the ordinary treatment constants are best replaced by

$$t_1', \quad t_2' - \frac{1}{p-2} t_1', \quad t_3' - \frac{1}{p-2} t_1', \dots,$$

with  $t_2' + t_3' + \dots = 0$ . With this artifice  $m$  still represents the arithmetic mean.

The equations for the constants now fall into groups of three, composed of one  $c$  equation, one  $r$  equation and one  $t'$  equation. The equation for  $t_1'$  stands by itself and is orthogonal with all the others.

The expressions for various sums of squares are as follows:

Rows: 
$$\frac{1}{(p-1)} (R_2^2 + R_3^2 + \dots) - \frac{1}{(p-1)^2} G^2.$$

Columns: 
$$\frac{1}{(p-1)} (C_2^2 + C_3^2 + \dots) - \frac{1}{(p-1)^2} G^2.$$

Treatments:

(1)  $v.$  rest:

$$\frac{1}{(p-1)^2 (p-2)} \{ (p-1) T_1 - G \}^2.$$

(2)  $v.$  (3)  $v.$  (4)  $v.$  ... :

$$\frac{1}{p(p-1)(p-3)} \{ [(p-1) T_2 + R_p + C_2]^2 + \dots \} \\ - \frac{1}{p(p-1)^2(p-3)} \{ (p+1) G - (p-1) T_1 \}^2.$$

The treatment comparisons are given by the differences of

$$\frac{T_1}{p-1}, \\ \frac{(p-1) T_2 + R_p + C_2}{p(p-3)} - \frac{2(p-1) G - 2T_1}{p(p-1)(p-2)(p-3)}, \\ \frac{(p-1) T_3 + R_{p-1} + C_3}{p(p-3)} - \frac{2(p-1) G - 2T_1}{p(p-1)(p-2)(p-3)},$$

The standard error of any pair of differences between these quantities is correctly given if we assign a standard error of

$$\sqrt{p \frac{p^2 - 4p + 2}{(p-2)(p-3)}} \times \text{the standard error of a single value}$$

to the first quantity, and a standard error of

$$\sqrt{\frac{(p-1)}{p(p-3)}} \times \text{the standard error of a single value}$$

to each of the others.

The case in which one row (or column) and one treatment are missing can be deduced from the case where one row and column are missing. The final sums of squares are as follows:

Rows: 
$$\frac{1}{p-1} (R_2^2 + R_3^2 + \dots) - \frac{1}{(p-1)^2} G^2.$$

Columns:

(1) *v.* rest:

$$\frac{1}{(p-1)^2(p-2)} \{(p-1) C_1 - G\}^2.$$

(2) *v.* (3) *v.* ...:

$$\frac{\{(p-1) C_2 + R_2\}^2 + \{(p-1) C_3 + R_3\}^2 + \dots - \{pG - (p-1) C_1\}^2}{(p-1)(p^2 - 3p + 1)} - \frac{\{pG - (p-1) C_1\}^2}{(p-1)^2(p^2 - 3p + 1)}.$$

Treatments:

$$\frac{\{(p^2 - 3p + 1) T_2 + (p-1) C_2 + R_2\}^2 + \dots - \{(p-1) G - C_1\}^2}{p(p-1)(p-3)(p^2 - 3p + 1)} - \frac{\{(p-1) G - C_1\}^2}{p(p-3)(p^2 - 3p + 1)}.$$

The total of these sums of squares is of course equal to the total of the sums of squares that would be obtained by interchanging columns and treatments in the expressions already given for the case of one missing row and column. In view of the greater complication of the above expressions this provides a useful check.

The treatment effects in units of a single value or "plot yield" are given by the differences between

$$\frac{(p^2 - 3p + 1) T_2 + (p-1) C_2 + R_2}{p(p-1)(p-3)},$$

etc., the standard error to be assigned to each being

$$\sqrt{\frac{p^2 - 3p + 1}{p(p-1)(p-3)}} \times \text{the standard error of a single value.}$$

The extremely small increase in standard errors in incomplete squares due to the unbalanced nature of the design may be illustrated by con-

sidering the case where one row is missing. The standard error of a treatment comparison is then

$$\sqrt{2} \times \sqrt{\frac{p-1}{p(p-2)}} \times \text{the standard error of a single value.}$$

The standard error that would be obtained in a randomized block experiment involving the same number of plots is

$$\sqrt{2} \times \sqrt{\frac{1}{p-1}} \times \text{the standard error of a single value.}$$

The ratio of the squares of these fractions,

$$\frac{p(p-2)}{(p-1)^2} = 1 - \frac{1}{(p-1)^2},$$

is the measure of the fraction of information lost when the error variances are the same in the two cases. Clearly this loss is likely to be far more than overbalanced by the gain due to the elimination of column effects in the design, provided the incomplete square is sufficiently large to give an adequate number of degrees of freedom for error.

#### ABSENCE OF BIAS IN ESTIMATE OF ERROR

The method of least squares on which the theory of the last section is based involves the assumptions that the experimental values are uncorrelated and are normally distributed. In reality neither assumption is true. In actual experimental work the process of randomization is so arranged that if the treatments produce no effect, then with any one set of experimental values the mean value of the error mean square in all the arrangements from which a random selection is made is equal to the mean value of the treatment mean square. It has been shown by actual trial<sup>(1)</sup> that in such cases Fisher's  $z$  distribution is a good approximation to the actual distribution of  $z$ . It has been further shown<sup>(4)</sup> that in the case of the Latin square one appropriate process of randomization is to take any square arrangement of plots fulfilling the conditions of the Latin square, and randomize all the rows except the first, and all the treatments.

A process of randomization which will equalise the mean values of the treatment and error mean squares does not always exist, and some otherwise admirable experimental arrangements fail on this criterion. There does not appear, for instance, to be any correct randomization process for the type of design which has been called the semi-Latin square. In one design of this type every treatment occurs once in each column

and once in each pair of rows, so that with  $p$  treatments there are  $\frac{1}{2}p^2$  plots. It is easy to see that sets of constants representing columns, treatments and pairs of rows are mutually orthogonal; consequently for values that are normally distributed and uncorrelated after allowing for the effects of these constants the ordinary procedure of the analysis of variance is valid. If, however, there is a residual correlation between pairs of plots in the same column and same row pair (a very likely event), processes of randomization applicable to a Latin square give decidedly biased estimates of the treatment mean square relative to the error mean square (5) (Discussion)).

When one row, column or treatment of a Latin square is missing it is by no means obvious that the estimates of error given by the least square method of the last section are unbiased when the original (or any other) process of randomization is used to determine the actual lay-out. We will now show that this is the case. For simplicity the proofs are given for a  $4 \times 4$  square; they are, however, perfectly general.

We will first take the case where one row is missing. Let the residuals after eliminating rows and columns only be denoted by  $x$  with the same suffixes as in the last section (treatment suffixes omitted). The full table of residuals will be

$x_{21}$	$x_{22}$	$x_{23}$	$x_{24}$
$x_{31}$	$x_{32}$	$x_{33}$	$x_{34}$
$x_{41}$	$x_{42}$	$x_{43}$	$x_{44}$

with the sum of each row and each column zero.

With the particular arrangement of treatments given in the last section the treatment sum of squares will be

$$\frac{1}{4 \cdot 3 \cdot 2} \left[ \{3(x_{22} + x_{33} + x_{44}) + (x_{21} + x_{31} + x_{41})\}^2 + \{3(x_{23} + x_{34} + x_{41}) + (x_{22} + x_{32} + x_{42})\}^2 + \dots \right].$$

Since  $x_{21} + x_{31} + x_{41} = 0$ , etc., this reduces to

$$\frac{3}{4 \cdot 2} [(x_{22} + x_{33} + x_{44})^2 + (x_{23} + x_{34} + x_{41})^2 + \dots].$$

On squaring out the brackets we obtain 12 products of pairs of residuals not in the same row or column. There are in all 36 such pairs of which the mean values can be represented by  $\overline{xx'}$ . It is easily seen that in all possible treatment arrangements given by the permutation of all the rows except the missing one of the original square each pair will occur

equally frequently in the treatment sum of squares. The mean value of the treatment sum of squares is therefore

$$\frac{3}{4 \cdot 2} \{12\overline{x^2} + 24\overline{xx'}\}.$$

Consider the products of  $x_{21}$  with the other residuals not in the same row or column. The total is

$$\begin{aligned} x_{21} \{ (x_{32} + x_{33} + x_{34}) + (x_{42} + x_{43} + x_{44}) \} \\ = x_{21} (-x_{31} - x_{41}) \\ = x_{21}^2. \end{aligned}$$

Taking the mean over all  $x$  we obtain  $6\overline{xx'} = \overline{x^2}$ . The mean value of the treatment sum of squares (3 degrees of freedom) is therefore  $6\overline{x^2}$ . The treatment-plus-error sum of squares (6 degrees of freedom) is  $Sx^2$ , or  $12\overline{x^2}$ , and therefore the mean value of the treatment mean square is equal to the mean value of the error mean square.

In the case where the values of one treatment are missing it will be most convenient to start with the residuals which are obtained by eliminating rows and columns when all the values exist. Let these residuals be

$x_{11}$	$x_{12}$	$x_{13}$	$x_{14}$
$x_{21}$	$x_{22}$	$x_{23}$	$x_{24}$
$x_{31}$	$x_{32}$	$x_{33}$	$x_{34}$
$x_{41}$	$x_{42}$	$x_{43}$	$x_{44}$

with the sum of each row and each column zero.

Consider the same arrangement of treatments as before, the values of treatment 1, *i.e.*  $x_{11}$ ,  $x_{22}$ ,  $x_{33}$  and  $x_{44}$ , being missing. Since

$$C_1 = x_{21} + x_{31} + x_{41} = -x_{11},$$

$$R_1 = x_{12} + x_{13} + x_{14} = -x_{11}, \text{ etc.}$$

and

$$G = -x_{11} - x_{22} - x_{33} - x_{44},$$

the sum of squares due to rows and columns is, from the formula of the last section,

$$\frac{1}{4 \cdot 2} \{ (x_{11}^2 + x_{22}^2 + x_{33}^2 + x_{44}^2) (3 + 2 + 3) - 2 (x_{11} + x_{22} + x_{33} + x_{44})^2 \}.$$

The mean value of this over all treatment arrangements generated by randomising the last three rows and randomizing treatment numbers is

$$\frac{1}{4 \cdot 2} (24\overline{x^2} - 24\overline{xx'}) = \frac{24}{9}\overline{x^2},$$

since  $\overline{xx'} = \frac{1}{3}\overline{x^2}$ .

The sum of squares due to treatments (2 degrees of freedom) is

$$\frac{1}{4} \{ (x_{12} + x_{23} + x_{34} + x_{41})^2 + (x_{13} + \dots)^2 + (x_{14} + \dots)^2 \} - \frac{1}{12} (x_{11} + x_{22} + x_{33} + x_{44})^2,$$

and the mean value of this is

$$\frac{8}{3} \overline{x^2} + 8 \overline{xx'} = \frac{32}{9} \overline{x^2}.$$

Similarly, the mean value of the total sum of squares is

$$\frac{35}{3} \overline{x^2} - \overline{xx'} = \frac{104}{9} \overline{x^2}.$$

The mean value of the treatment-plus-error sum of squares (5 degrees of freedom) is therefore  $\frac{80}{9} \overline{x^2}$ , and of the corresponding mean square is  $\frac{16}{9} \overline{x^2}$ , which is the same as for treatments.

The cases where one row and one column are missing, and one row and one treatment, follow exactly the same lines, and bias is again found to be absent. There is no need to reproduce the details of the algebra here.

### EXAMPLES

*Example 1.* Fig. 1 gives the plan and yields of roots of a sugar-beet experiment at Tunstall in which five different levels (0, 1, 2, 3, 4 tons per acre) of chalk were applied. The yields at the lowest level were only

Plan and yields of roots in lb.  
(plot area: 0.0168 acre)

	3 702	1 571	0 [89]	4 722	2 710	<i>R</i> 2705	4 <i>R</i> + <i>C</i> 13216	<i>R</i> + <i>C</i> - 3 $y_0$ 4834
0 [82]		2 668	4 667	1 546	3 676	2557	12854	4937
4 675		3 612	1 554	2 575	0 [126]	2416	12108	4482
1 636		0 [53]	2 589	3 573	4 583	2381	11981	4679
2 613		4 606	3 586	0 [52]	1 475	2280	11536	4540
<i>C</i>	2626	2457	2396	2416	2444	12339 616.95	61695 12339	23472 4694.4
Treatment:		0	1	2	3	4		
Total:		[402]	2782	3155	3149	3253		

Fig. 1. Sugar-beet, Tunstall, 1932.

about 15 per cent. of the mean yield and there can therefore be no real ground for assuming that the errors affecting these plots, or the row and column differences, are the same as for the remainder of the experiment.

The row, column and treatment totals, omitting the zero dressing, are given in the figure, together with the quantities  $4R+C$ . Here row 1 is associated with column 3 so that the first of these quantities is  $4 \times 2705 + 2396 = 13,216$ . The sum of the squares of the deviations is divided by 5.4.3 or 60. This gives the sum of squares 31,011.

The full analysis of variance is given in Table II, and for comparison the analysis including the zero dressing is also given. The error mean square is reduced from 1824 to 1507 by the omission of the zero dressing. This does not imply that the errors of the rejected plots are greater than those of the other plots, since differences in the magnitude of the row and column effects at the two levels of yield also contribute to the apparent errors in the analysis of the whole square.

Table II. *Analysis of variance; Tunstall sugar-beet*

	Omitting untreated plots			Including all plots		
	Degrees of freedom	Sum of squares	Mean square	Degrees of freedom	Sum of squares	Mean square
Rows	4	31,011	7,753	4	25,697	6,424
Columns	4	8,387	2,097	4	7,579	1,894
Treatments	3	25,806	8,602	4	1,177,349	294,337
Error	8	12,055	1,507	12	21,890	1,824
Total	19	77,259	—	24	1,232,515	—

The difference between the two error sums of squares is calculable directly from the differences of the quantities  $R+C-3y_0$ . Their values are given in Table II. The sum of the squares of the deviations, divided by  $5 \times 3$  or 15, is 9836, agreeing with the analyses. This sum of squares corresponds to 4 degrees of freedom, giving a mean square of 2459, which is formally comparable with the other error mean square 1507. If the levels of yield were similar it would give a test of whether the depression due to absence of chalk was different on the different parts of the experiment.

*Example 2.* Fig. 2 gives the yields of a swede experiment involving different types of phosphate. The original design consisted of a  $6 \times 6$  Latin square, but six of the eight rows of plants in the first column failed and were resown with another variety, which was inadvertently lifted by the farmer without weighing. In addition one plot, marked



with an asterisk, was badly damaged by crows, and the plots without fertiliser gave yields of about a sixth those of the other plots. The yields from all these plots have therefore been rejected in the analysis.

The yield of the plot damaged by crows has been estimated by means of the missing plot technique (3). The row, column and treatment totals shown in the round brackets are those obtained before estimation of the

Plan and yields of roots in units of 10 lb.  
(plot area 1/40 acre)

<i>B</i> —	<i>W</i> 109*	<i>G</i> 90	<i>Re</i> 90	0 [4]	<i>Y</i> 115	404 (295)	5 <i>R</i> + <i>C</i> 2636
<i>G</i> —	<i>B</i> 122	<i>Y</i> 134	0 [18]	<i>W</i> 126	<i>Re</i> 99	481	3005
0 —	<i>Y</i> 131	<i>B</i> 126	<i>W</i> 136	<i>Re</i> 106	<i>G</i> 114	613	—
<i>Re</i> —	<i>G</i> 112	0 [26]	<i>B</i> 129	<i>Y</i> 138	<i>W</i> 121	500	3095
<i>W</i> —	0 [26]	<i>Re</i> 106	<i>Y</i> 135	<i>G</i> 120	<i>B</i> 122	483	2990
<i>Y</i> —	<i>Re</i> 101	<i>W</i> 139	<i>G</i> 110	<i>B</i> 126	0 [17]	476	2951
	575 (466)	595	600	616	571	2957 (2848)	14677
Total ( <i>T</i> )	0 [91]	<i>Re</i> 502	<i>G</i> 546	<i>B</i> 625	<i>Y</i> 653	<i>W</i> 631 (522)	Total 2957
19 <i>T</i> + 5 <i>R</i> + <i>C</i>	—	12633	13379	14511	15358	14979	70860

Fig. 2. Swedes, Burn, Ayrshire, 1934.

missing plot. In order to make the estimate we denote the missing yield by  $x$  and evaluate the error sum of squares as if  $x$  were known. The required estimate will be the value of  $x$  which makes this sum of squares a minimum.

There is no need at this stage to include any terms not involving  $x$ , as these will disappear on differentiation. The computation is also simplified by using the expressions developed for a missing row and column (substituting treatments for rows), for as already pointed out

the error sum of squares will be identical with that given by the more complicated expressions for a missing column and treatment.

The sums of squares so obtained are as follows.

Rows: 3 v. rest:

$$\frac{1}{100} (5 \times 613 - 2848 - x)^2.$$

Rows: 1 v. 2 v. 4 v. 5 v. 6:

$$\frac{1}{90} (5 \times 295 + 5x + 616 + 625)^2 + \frac{1}{90} (5 \times 483 + 522 + x + 466 + x)^2 \\ - \frac{1}{450} (7 \times 2848 + 7x - 5 \times 613)^2.$$

$$\text{Columns:} \quad \frac{1}{8} (466 + x)^2 - \frac{1}{25} (2848 + x)^2.$$

$$\text{Treatments:} \quad \frac{1}{8} (522 + x)^2 - \frac{1}{25} (2848 + x)^2.$$

$$\text{Total:} \quad x^2 - \frac{1}{25} (2848 + x)^2.$$

This gives for the error sum of squares

$$\frac{375}{900} x^2 - \frac{41025}{900} (2x) + \text{a constant.}$$

Differentiating and equating to zero we obtain

$$x = \frac{41025}{375} = 109.4.$$

This value can be inserted in the table of results and the row, column and treatment totals modified to include it, as shown by the unbracketed totals.

We can now proceed with the main analysis. Row 1 is associated with treatment B and column 5, etc. Row 3 stands on its own as it contains all the treatments.

The results are given in Table III. The sum of squares for columns is obtained direct from the column totals. The sum of squares for all rows except 3 is the sum of squares of the deviations of the quantities  $5R + C$  (shown in the table) divided by 95, while the sum of squares for row 3 v. the rest is  $(5 \cdot 613 - 2957)^2/100$ . The sum of squares for treatments is the sum of squares of the deviations of  $19T + 5R + C$  (also shown in the table) divided by 1710.

Table III. *Analysis of variance; Burn, Ayrshire, Swedes Experiment*

	Degrees of freedom	Sum of squares	Mean square
Rows	{ 1	116.64	
	{ 4	1296.64	
Columns	4	275.44	
Treatments	4	3023.47	755.87
Error	10	234.85	23.48
Total	23	4947.04	

The degrees of freedom due to error must be reduced by one on account of the missing plot. This gives a mean square error of 23.48.

The treatment effects on a single plot basis are measured by  $(19T + 5R + C)/90$ , namely

<i>Re</i>	<i>G</i>	<i>B</i>	<i>Y</i>	<i>W</i>
140.4	148.7	161.2	170.6	166.4'

the appropriate standard error of each quantity except *W* being

$$\sqrt{\frac{19}{90} \times 23.48} \text{ or } \pm 2.23.$$

The analysis of the experiment as one of five randomized blocks (ignoring the rows) would have led to a very decided over-estimate of the error owing to the lower values of row 1. The actual estimate obtained (if the value 109 for the missing plot is taken as a real value) is 85.16. By rejecting this row, however, and treating the remainder of the experiment as a simple random arrangement without rows or columns a very good estimate of the error is obtained, namely 23.00, owing to the fact that the other rows and columns eliminate no appreciable amount of the variance. The full analysis already given, however, is by no means laborious (except for the determination of the missing value), and can reasonably be undertaken in cases such as this.

The standard error of *W* is increased owing to the one missing value. In the case of a complete square it has been shown (3) that the increase is in the ratio of  $\sqrt{(p^2 - 2p + 2)/(p - 1)(p - 2)}$ , this giving a ratio of 1.19 for  $p = 5$  and of 1.14 for  $p = 6$ . The actual increase is unlikely to be appreciably greater than the first of these values.

### SUMMARY

A description is given of the statistical procedure appropriate for the analysis of a Latin square having missing the whole of one row, one column or one treatment, or one row and one column, or either and a treatment. These are the only types of incomplete Latin squares (except those which can be dealt with by the missing plot technique) for which a neat statistical analysis is possible.

It is shown that incomplete Latin squares of these types give unbiased estimates of error and are therefore valid experimental arrangements. They are consequently likely to be of use when the experimental material is such as to preclude the use of a complete Latin square owing to the fact that the number in one or both of the natural groups is one less than the number of treatments to be tested.

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# INVESTIGATIONS IN CROP HUSBANDRY

## III. EFFECT OF TIME OF APPLICATION OF SULPHATE OF AMMONIA TO WHEAT

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(With One Text-figure)

At the beginning of the century it was authoritatively predicted that, in the absence of the commercial fixation of atmospheric nitrogen, there would be a world shortage of wheat after 1930. It would be difficult to argue that the present actual surplus is entirely due to the fact that atmospheric nitrogen has been commercially exploited, but the prediction serves to show the importance of nitrogen in the growing of wheat. That nitrogenous manures are widely used is shown by figures collected in the eastern counties of England for the agricultural year 1930-1; despite the fact that conditions were very depressed and that 40 per cent. of the wheat acreage received farmyard manure, 22 per cent. of the crop received quick-acting nitrogenous fertilisers, the average dressing being rather over 1 cwt. to the acre. It is certain that at the present time, with an enhanced price for wheat and the extension of mechanisation, a greater proportion of the crop receives a nitrogenous dressing. It is obviously important to determine the optimum time for the application of this dressing, and experiments with that object in view, for the case of autumn-sown wheat, have been carried out at Cambridge during the last six years.

Some of the classical plots on Broadbalk Field (continuous autumn-sown wheat) at Rothamsted have thrown light on the problem; over a series of years spring applications of sulphate of ammonia gave higher returns than autumn applications owing to the leaching of the latter during wet winters. Shortly after the War the work of Engledow<sup>1</sup> and others shed further light on the problem. It was shown that autumnal nitrogenous dressings markedly increased the production of early, *i.e.* potential ear bearing, tillers, and that dressings in late spring increased the size but not the number of ears. It was therefore suggested that a

<sup>1</sup> See *J. agric. Sci.* (1929), **19**, 472.

good procedure would be to apply half the dressing early and half late so that both the above beneficial effects on grain yield might be obtained.

The effect of nitrogenous dressings is highly dependent on the richness of the land to which they are applied. In the enquiry to be described three experiments were located upon the light land on the University Farm which has been highly manured for a number of years; this land is in very good heart, and the effect of added nitrogen was invariably harmful. It was therefore decided to select fields in a low state of fertility for the subsequent work. The selection of poor land for manurial trials is generally open to criticism, but where differential time effects are sought it is essential that some response should be obtained. The fields used were suitable for growing wheat, but for some years previous to their acquisition by the University Farm they had been poorly farmed.

For the sake of simplicity only one nitrogenous fertiliser was used, and the one selected was sulphate of ammonia as that is the form of nitrogen most commonly employed. A further series of experiments has been initiated in which it is hoped to include other forms of nitrogen.

#### THE EXPERIMENTS

During the years 1929-31 three experiments were conducted, two being on light land in high condition, and one on heavy clay in good heart. In each case the manurial investigation was superimposed on a cultivation experiment, the plots of which were split for its inclusion; although the total area concerned was large only a few treatments could be compared. It was therefore considered desirable to restrict the enquiry to early dressings, using a February application as a standard representing normal practice. The four manurial treatments were:

- A.  $\frac{1}{2}$  cwt. at seeding and  $\frac{1}{2}$  cwt. in February.
- B.  $\frac{1}{2}$  cwt. in December and  $\frac{1}{2}$  cwt. in February.
- C. 1 cwt. in February.
- D. No manure.

This scheme was far from perfect, and experience soon showed that the comparisons were not sufficiently clear cut. It was therefore decided to have three widely separated times of application and to include single and all possible combinations of half-dressings; this necessitated divorcing the enquiry from the cultivation experiments, and for the next four years the following scheme remained unchanged:

- (1) Whole dressing at seeding.
- (2) Whole dressing in February.

- (3) Whole dressing in May.
- \* (4) Half dressing at seeding and half-dressing in February.
- (5) Half-dressing at seeding and half-dressing in May.
- (6) Half-dressing in February and half-dressing in May.
- (7) No manure.

These four experiments took the form of Latin squares, with small plots. Each row of a Latin square was a drill width (7 ft. 6 in.), and columns were formed by pathways 2 ft. wide running perpendicular to the direction of drilling and 17 ft. apart. The drill sowed twelve lines of seed of which two at each side were discarded; further discards of 1 ft. on each side of each pathway were allowed, so that the ultimate plot was 1/581 acre. It must be admitted that this is very small for field work, but the arrangement has proved convenient in practice and considerable accuracy in working can be achieved, as all harvesting operations are performed by hand; the lay-out has been justified by the smallness of the experimental errors obtained.

The first year the Latin square lay-out was employed the experiment was again situated on rich light land and a full dressing was at the rate of 1 cwt. per acre; in the last three years, as already mentioned, poorer land was selected and the rate of application raised to 1½ cwt. per acre.

Table I. *Details of experimental fields*

Year	Soil type	Previous crop	Mean yield		Variety of wheat	Remarks
			Grain (bushel per acre)	Straw (cwt. per acre)		
1929-30	Light	Potatoes	27.75	65.50	Little Joss	Winter proud. Very badly laid by thunderstorm in June
1930-31	Light	Potatoes	45.60	32.51	Little Joss	Some lodging and some loss from "foot-rot"
1930-31	Heavy	Beans	32.97	37.44	Wilhelmina	Some lodging but cut with binder
1931-32	Light	Potatoes	19.78	—	Yeoman II	Very bad attack of "foot-rot"
1932-33	Heavy	Beans	45.58	51.88	Wilhelmina	Stood well at harvest
1933-34	Heavy	Mangolds	60.88	52.85	Wilhelmina	Stood well at harvest
1934-35	Heavy	Wheat	45.40	—	Wilhelmina	Stood well at harvest

Table I gives the agricultural details of the experiments. Of the three light-land experiments only one gave a satisfactory yield; in 1929-30 the crop was very thick and became winter proud, the wet spring accentuating the trouble and preventing remedial measures, whilst in 1931-2

the crop was ruined by a severe attack of "foot-rot". The summer of 1931 was very wet and therefore unfavourable to wheat on heavy land, whilst the last three years have been very favourable; in East Anglia 1933-4 was one of the best wheat years of the century, and this experimental field was no exception in yielding much more highly than was expected. Straw weights have been obtained by subtracting the grain weights from the sheaf weights at threshing time; in 1931-2 and 1934-5 sheaves were not weighed. It will be observed that the mean yields shown in this table are not in conformity with the remarks made above as to richness of land; the discrepancies were clearly due to lodging, disease and favourability of the season.

#### DEVELOPMENTAL COUNTS

In a study of this nature it is obviously important to make a series of counts and observations during growth in order to detect the effect of the treatment on the development of the crop. In all cases the sampling unit was a one foot length of drill row, but various methods of dispersion of the units over the plots have been used. With the Latin square lay-out described above bridges can be used to span the plots and counts can be made without trampling; this method has proved very satisfactory in operation, and two random foot lengths have been taken from each drill row giving sixteen foot lengths per plot. When the weather permitted, one or two germination counts and several stem and plant counts were made. In Tables II and III the results of three counts only are given for each experiment. The germination count was made within a few days of December 10 (in 1933-4 it had to be postponed to January 13), the early stem counts were made in the second half of February, and the late stem counts about the middle of April. Wet weather prevented the stem count in February 1931, and both the germination and early stem counts in 1935; in these cases it was considered better to lose the count rather than to ruin the crop, because even with bridges some harm is done to the crop under wet conditions owing to the extreme puddling of the discards. In field work, where a large number of units have to be dealt with, it is found better to count all the stems rather than to try to differentiate between main stem and side tillers.

For simplicity of presentation the results are shown in two tables, the division being according to the manurial scheme used.

Apparently the application of sulphate of ammonia immediately after drilling has no effect upon germination and plant establishment. It will be realised that with all the pre-harvest counts shown there was more



than one control treatment; the principle has been to compare all treatments which had already received nitrogen with those that had not.

Table II. *Summary of results of three experiments*

Year	Soil	Half seeding, half Feb. A	Half Dec., half Feb. B	Whole in Feb. C	No manure D	s.e. of relative means	Significance
(a) Germination count							
1929-30	Light	105.4	105.0	107.1	100.0	3.08	Insignificant
1930-31	Light	93.4	95.8	92.6	100.0	2.69	Insignificant
1930-31	Heavy	100.4	98.7	102.0	100.0	3.09	Insignificant
(b) Early stem count							
1929-30	Light	109.8	113.5	104.0	100.0	3.66	(ABC) > D*
1930-31	Light	100.6	96.9	97.5	100.0	3.42	Insignificant
1930-31	Heavy	—	—	—	—	—	—
(c) Late stem count							
1929-30	Light	112.5	115.0	107.7	100.0	3.53	(ABC) > D†
1930-31	Light	98.5	96.8	101.5	100.0	4.33	Insignificant
1930-31	Heavy	109.3	114.4	107.7	100.0	4.56	Insignificant
(d) Ear number							
1929-30	Light	—	—	—	—	—	—
1930-31	Light	105.3	104.4	107.4	100.0	3.22	Insignificant
1930-31	Heavy	112.3	108.6	107.1	100.0	3.45	(ABC) > D*
(e) Corrected ear weight							
1929-30	Light	—	—	—	—	—	—
1930-31	Light	91.6	92.1	89.9	100.0	2.62	D > (ABC)†
1930-31	Heavy	96.4	95.8	96.5	100.0	1.03	D > (ABC)†
(f) Yield of grain							
1929-30	Light	88.0	91.8	90.1	100.0	2.47	D > (ABC)†
1930-31	Light	82.3	82.7	89.7	100.0	3.75	D > (ABC)†
1930-31	Heavy	105.3	100.9	103.7	100.0	2.76	Insignificant
(g) Yield of straw							
1929-30	Light	106.2	99.0	106.9	100.0	3.48	Insignificant
1930-31	Light	101.2	100.9	108.2	100.0	3.14	Insignificant
1930-31	Heavy	106.2	103.6	106.4	100.0	3.72	Insignificant

\* Significant ( $P < 0.05$ ).

† Significant ( $P < 0.01$ ).

(ABC) > D denotes that the mean of treatments A, B, C was significantly higher than that of treatment D.

It seems that the early application of nitrogen first produces an effect on tiller formation some time in February. It is true that only two out of five of the early stem counts showed significant increases, but three of these counts refer to rich land; of the three experiments on poor land one gave a significant result, one just missed significance, whilst on the third no count was possible.

Table III. *Summary of results of four experiments*

Year	Soil	Whole seeding 1	Whole Feb. 2	Whole May 3	Half seeding half Feb. 4	Half seeding half May 5	Half Feb., half May 6	No manure 7	S.E. of relative means	Significance
(a) Germination count										
1931-32	Light	99.5	88.2	92.1	95.9	95.9	100.9	100.0	3.82	Insignificant
1932-33	Heavy	112.7	85.7	101.5	88.0	101.9	83.7	100.0	5.37	Insignificant
1933-34	Heavy	100.4	79.7	89.2	102.3	97.7	89.4	100.0	5.64	Insignificant
1934-35	Heavy	—	—	—	—	—	—	—	—	—
(b) Early tiller count										
1931-32	Light	91.4	86.0	89.2	89.6	89.2	94.2	100.0	3.72	Insignificant
1932-33	Heavy	114.2	90.4	105.9	95.9	107.0	84.7	100.0	5.75	(145) > (2367)*
1933-34	Heavy	109.9	83.7	90.6	100.7	102.2	93.6	100.0	6.13	Insignificant
(c) Late tiller count										
1931-32	Light	89.5	91.4	87.0	90.0	84.4	94.8	100.0	3.89	Insignificant
1932-33	Heavy	113.6	104.2	101.2	104.1	106.2	96.9	100.0	3.96	(145) > (2367)*
1933-34	Heavy	129.0	107.9	93.6	122.7	119.0	105.0	100.0	5.33	(145) > (2367)†
1934-35	Heavy	119.4	130.3	106.8	123.5	122.1	122.1	100.0	6.74	(12456) > (37)*
(d) Ear number										
1931-32	Light G	98.2	114.9	122.7	86.1	87.5	95.7	100.0	7.76	(236) > (145)†
	T	97.1	99.1	99.2	89.3	90.5	102.9	100.0	3.93	(236) > (145)*
1932-33	Heavy	122.3	114.3	112.3	118.3	119.9	110.0	100.0	3.58	(123456) > (7)†
										(145) > (236)*
1933-34	Heavy	126.3	122.2	107.9	127.2	116.0	113.6	100.0	2.33	(12456) > (37)†
1934-35	Heavy	98.6	97.9	104.7	96.9	96.5	103.4	100.0	2.43	Insignificant
(e) Corrected ear weight										
1931-32	Light G	99.1	81.4	83.1	92.1	100.8	84.7	100.0	5.53	Insignificant
	T	100.2	95.5	99.4	87.8	97.2	88.1	100.0	6.43	Insignificant
1932-33	Heavy	101.1	102.2	106.4	107.0	110.1	107.3	100.0	2.95	(356) > (124)*
1933-34	Heavy	96.2	100.1	101.8	93.5	100.0	100.1	100.0	1.61	Insignificant
1934-35	Heavy	105.0	105.9	110.1	100.1	112.7	106.9	100.0	3.33	(356) > (124)*
(f) Yield of grain										
1931-32	Light G	97.2	96.4	105.9	78.0	88.1	80.3	100.0	9.58	(123) > (456)*
	T	99.1	95.2	99.1	84.1	93.6	89.3	100.0	6.41	Insignificant
1932-33	Heavy	117.2	112.6	118.1	120.8	125.4	115.2	100.0	4.10	(123456) > (7)†
1933-34	Heavy	125.2	125.1	111.1	123.0	118.0	116.1	100.0	3.09	(124) > (356)*
										(356) > (7)*
1934-35	Heavy	104.2	104.9	111.4	98.8	111.3	109.2	100.0	3.37	(356) > (124)*
(g) Yield of straw										
1931-32	Light	—	—	—	—	—	—	—	—	—
1932-33	Heavy	119.5	117.6	119.3	121.4	123.9	118.1	100.0	3.69	(123456) > (7)†
1933-34	Heavy	128.3	127.0	105.2	126.0	115.9	111.9	100.0	3.20	(124) > (356)†
										(356) > (7)†
1934-35	Heavy	—	—	—	—	—	—	—	—	—

\* Significant ( $P < 0.05$ ).† Significant ( $P < 0.01$ ).

(145) &gt; (2367) denotes that the mean of treatments 1, 4 and 5 was significantly higher than the mean of the treatments 2, 3, 6 and 7.

That the effect of nitrogenous dressings applied early is to lead to a greater number of tillers by the middle of April appears to be a justifiable conclusion. Even on the light land (already rich in nitrogen) there was a significant increase in one year out of three; on the poor heavy land the differences were marked and significant in all three cases, whilst on the heavy land in good heart the difference just failed to attain significance.

It should be observed that it was the seeding dressings<sup>1</sup> which gave the increases in April and that the effect of February dressing was only detectable in 1929-30 and in 1934-5.

#### HARVEST RESULTS

With the large plots used in the first three experiments harvesting was effected with a binder, the sample foot lengths having first been taken out (except in 1929-30 when the crop was very badly lodged); the samples were only used to determine ear number and ear size, the yield of grain and straw shown being that obtained from the whole plots. With the later Latin square arrangements, the plots being smaller, much greater care could be exercised in harvesting; the whole plots were cut by hand and threshed in a small-scale thresher, every ear being counted.

Lodging occurred on four of the experiments. In 1929-30 and in 1931-2 nearly the whole area was laid flat, a storm being responsible in the former year and "foot-rot" in the latter, and some lodging occurred in 1930-1 on heavy land; in none of these cases was it possible to detect any effect of nitrogen on lodging. On the light land in 1930-1 some lodging occurred shortly before harvest, and the amount on each plot was assessed numerically; the figures were, of course, merely estimates, but treated statistically they showed that all nitrogenous dressings had increased lodging, the increase being greatest where a half-dressing had been applied at seeding or in December.

In two of the experiments "foot-rot" played a part. On the light land in 1930-1 its ravages were not serious, but some ears ripened prematurely and contained little or no grain at all or only a few shrivelled grains. The proportion of diseased ears was estimated for each plot prior to cutting, and statistical analysis of the figures obtained showed that nitrogen had very significantly increased the incidence of disease, irrespective of when it was applied. In 1931-2 the attack was much more severe. Many ears ripened prematurely and contained shrivelled grain, and these ears showed up black owing to secondary infection of *Cladosporium herbarum*. At threshing diseased ears were counted and threshed separately, and thus the true proportion of diseased ears could be calculated separately for each plot; in this case the effect of nitrogen was not itself significant, but the proportion of diseased ears was significantly higher where two half-dressings had been applied than where all the nitrogen was applied at one dressing. The result is somewhat confused by the fact that the

<sup>1</sup> This expression is used to refer to manurial application at the time of drilling and, of course, is in no way connected with seed treatment.

control treatment was intermediate. In Table III the results for 1931-2 for ear number, corrected ear weight and yield of grain are given both for the good ears (G) and for all ears (T); this was considered necessary because the grain from the diseased ears was very shrivelled and was chiefly tail corn.

It might be expected that the placing in regard to number of ears would be in conformity with that for the stem counts; a perusal of Tables II and III shows less agreement than might be anticipated. On the light land the failure to obtain ear numbers in 1929-30 is regrettable because in that year nitrogenous dressings had undoubtedly increased tiller number; in 1930-1 no significance is shown for stems or ear number, but it will be noted that a reversal occurred, whilst in 1931-2 there was no correspondence at all. On heavy land, by contrast, in 1930-1, 1932-3 and 1933-4 the agreement between stem and ear numbers was close, but in 1934-5 the differences as regards late stems were highly significant, whereas there was no appreciable difference in ear number. It is impossible to draw any conclusion as to the relative merits of early and late stem counts for forecasting ear number. The suggestion made by Engledow that early nitrogenous dressings increase ear numbers was only borne out in two years (1932-3 and 1933-4), by comparison of early and late dressings, and in 1930-1 on heavy land by comparison with no dressing; it was negatived in the 1931-2 experiment in which "foot-rot" played so large a part.

Statistical methods are now available for determining the effects of treatments on one series of observations after making due allowance for variations in an earlier series; this method (*i.e.* the covariance) was used to determine corrected ear weight (*i.e.* weight of ear corrected for number of ears). On the light land the only indication is that nitrogenous dressings decrease ear size. In 1930-1 all dressings were early and the effect was highly significant; in 1931-2, whilst no significant differences were shown, the trend of the figures was indicative of decreased ear size for all nitrogenous dressings, and this was probably associated with the incidence of "foot-rot". On the heavy land significance as regards ear size was achieved in three out of four cases. In 1932-3 and 1934-5 the May dressings definitely increased ear size whilst in 1930-1, when all nitrogenous dressings were early, ear size was decreased. Thus the general conclusion is favourable to Engledow's suggestion that late nitrogenous dressings increase ear size.

It has already been said that the light land on the University Farm is in good heart, and it will be seen that the effect of added nitrogen on that soil in every case was to decrease yield. In the first two years the results

were highly significant, and whilst in 1931-2 there was no significant difference in total yield the control plots gave the highest figure. Of the two years in which ear number, and thence ear size, were obtained in one case the decreased yield was definitely due to decreased ear size, whilst in the other it was apparently chiefly due to the same cause. On the heavy land, which has been well farmed for some years (1930-1 experiment), there was some slight increase in yield from nitrogenous dressings, but the result was quite insignificant. On poor heavy land, however, results more favourable to nitrogenous dressings emerged. In 1932-3 and 1933-4 nitrogen significantly increased yield by 15 to 20 per cent., whilst in 1934-5, though the difference between the mean of all nitrogenous dressings and that of the control was just insignificant, the increase was over 5 per cent.; reference to Table I will show that, though the land was poor, good results were obtained in these three experiments, and this may be partially due to favourable seasons. As regards the comparison of the times of application of the nitrogen there were marked differences in the three years. In 1932-3 no difference between times of application could be detected; in 1933-4 the early dressings were definitely better than the late, and in 1934-5 the late were definitely better than the early. These seasonal discrepancies will be further discussed below.

In the two experiments on light land in which the straw was weighed there was no significant increase associated with nitrogenous dressings, and in the 1930-1 experiment on heavy land the small increase obtained was not significant. In the two experiments on poor land in which the straw was weighed the results closely paralleled those for grain, the significance in the case of straw being rather more definite.

#### DISCUSSION OF RESULTS

For some years it has been recognised that care is called for in regard to nitrogenous manuring; whilst large increases in yield may usually be expected it is known that on rich land harm may result. There has been some tendency in the past to stress the benefits rather than to warn of the dangers. It is therefore salutary to observe that in each of the three experiments conducted on light land nitrogenous dressings decreased yield, this result being apparently due chiefly to its favouring lodging and disease. It is true that those three experiments followed potatoes and that the fields had been highly manured for some years; nevertheless the soil was shallow and gravelly and not naturally fertile.

On heavy clay soil the results have been much more favourable to nitrogenous dressing, significant results having been obtained in all four cases. Of these four experiments the first (1930-1) stands rather apart as

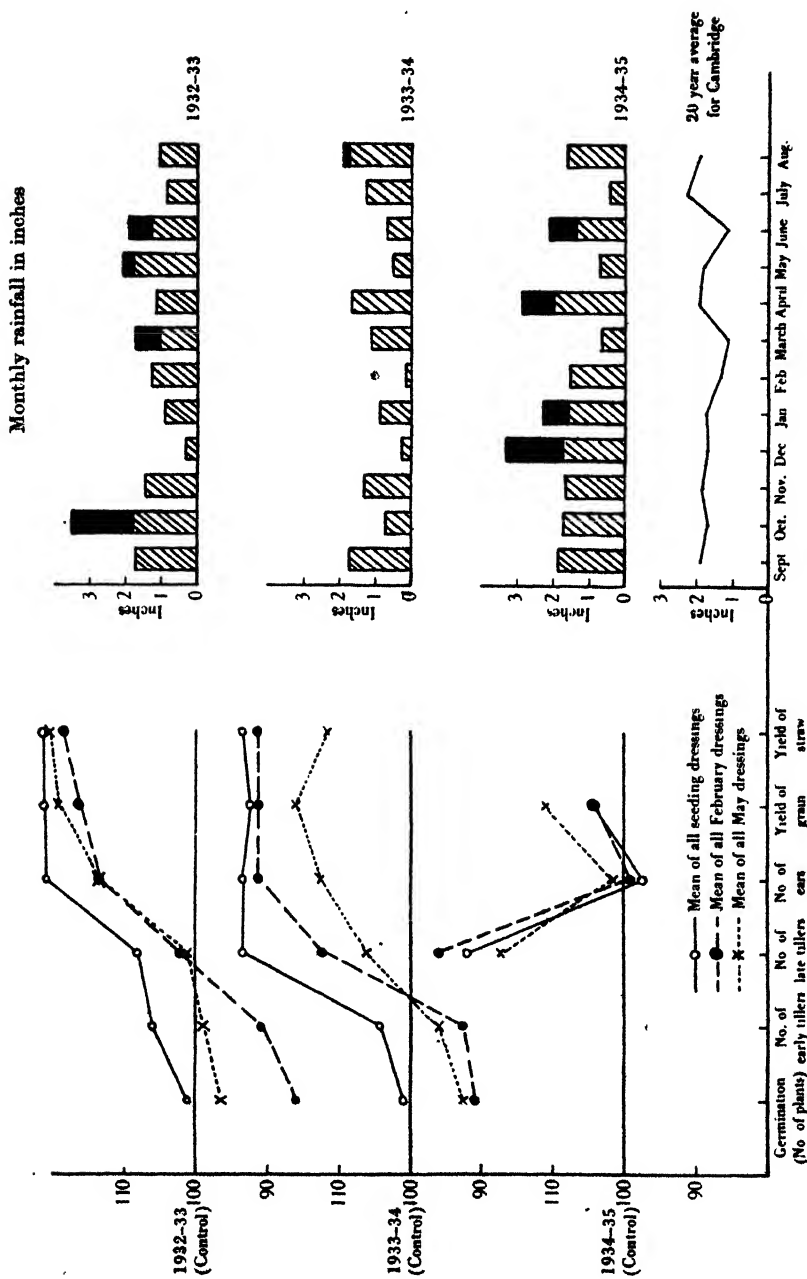


Fig. 1. Figures for seeding, February and May dressings with "no manure" as 100.

including a different manurial plan and as being located on land in better condition; in this experiment all dressings were applied before the end of February, and the comparisons were not wide enough to detect differences between times of application. The last three experiments were closely comparable in treatments, lay-out and soil type, and the results are illustrated in Fig. 1. In this figure an attempt has been made to differentiate between applications at seeding, in February and in May, but it must be realised that some confusion arises because of the fact that two half-dressings were applied in some treatments. For each year and each observation the mean for the control plots is taken as 100; for the May dressings the relative means of treatments 3, 5 and 6 are shown, whilst for February dressings those for treatments 2, 4 and 6 and for seeding dressings those for treatments 1, 4 and 5, are shown. This overlapping is regrettable but cannot be avoided, and this accounts for the fact that in some instances apparent responses are shown earlier than the time of application. The figure also shows the rainfall month by month for each year. The actual figures for rainfall were obtained in Cambridge, and the figure at the foot shows the normal for Cambridge over the last twenty years; when the rainfall in any month exceeded the normal the excess is shown as a black column. The agricultural year 1932-3 was characterised by a wet October and some excess rainfall in March, May and June, the remaining months, particularly December and January, being drier than normal. In that year the dressings at seeding produced a response in the early stages and slightly (insignificant) higher yields. It is shown in Table III that May dressings increased ear size in 1932-3, as is of course shown by the figure; thus when May and June were rather wet May dressings were effective. 1933-4 was a very abnormally dry year throughout, and it is very clear that early dressings were much more effective than May dressings. In that very dry summer the response of ear weight to May dressings was smaller and insignificant, and in that year the early dressings were undoubtedly more effective. In 1934-5 the months December, January, April and June were considerably wetter than normal. The wet winter rendered the seeding dressing ineffective, presumably through leaching, whereas the wet June produced a large response from the May dressing which markedly increased ear size.

It is clear that the relative merits of these three times of application depend upon the weather of the particular season. Where the winter is dry seeding dressings produce the largest increase in yield, whereas with a wet winter most of such dressing is lost. May dressings are particularly effective after a wet winter, and provided there is sufficient rainfall after their application to wash them in. A general conclusion in agreement with

Engledow's findings as to the effect of nitrogen at various stages of growth may be drawn, though in practice the responses will depend highly on the weather.

What practical advice for the farmer arises from these experiments? Where any particular time proved most effective it was almost immaterial whether the whole or the half-dressing was applied; it would therefore appear desirable to apply half the nitrogenous manure at seeding and to save the other half for May. This recommendation is not made on the basis that the former will increase ear number and the latter ear size (though that in fact will be the effects), but on the grounds that one of the applications will be effective, the weather determining which one. The common practice in this country is to apply the whole dressing in February, and this might be regarded as a reasonable compromise, but in these experiments it has never given the highest yield.

#### SUMMARY

1. Over a period of six years seven field experiments were carried out to study the effect of the time of application of sulphate of ammonia to autumn-sown wheat.

2. Three experiments were located on light gravelly soil which had been farmed highly for some years, and in those three cases sulphate of ammonia decreased yield, irrespective of time of application; the reduction in yield was of the order of 10 per cent. and is ascribed to more lodging and greater incidence of "foot-rot".

3. Three experiments were located on heavy clay soil in poor condition; in these sulphate of ammonia gave percentage increases in yield of 18, 20 and 7.

4. Evidence is produced that early dressings of sulphate of ammonia do not affect germination or plant establishment, but that they tend to increase tiller formation by the end of February.

✓ 5. Early dressings tend to increase the number of ears at harvest, whilst late (May) dressings tend to increase ear size.

6. The optimum time of application will depend entirely on the weather of a particular season. When the winter is wet autumn dressings are ineffective, whilst May dressings are very beneficial provided that the June rainfall suffices to wash them in; when the winter is dry early dressings are more effective than late.

7. It is recommended that half the dressing be applied at seeding time and that the other half be reserved for May application.

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# NOTES ON THE OSMOTIC PRESSURE OF THE CONTENTS OF THE STOMACH COMPARTMENTS OF THE SHEEP

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THE sheep possesses four so-called stomachs—the reticulum, the rumen, the omasum and the abomasum. The first three are developments of the caudal end of the oesophagus and only the fourth is comparable with the stomach of non-ruminant mammals. The rumen contains an extensive flora and fauna of cellulose-splitting bacteria and symbiotic ciliates and it, together with the reticulum, is a chamber where cellulose digestion and mixing of food takes place. The processes involved in the digestion of cellulose are incompletely known, but it can be regarded as definite that the contents of the four chambers consist of more than a mixture of cellulose and digestive juices: glucose and fatty acids are possibly derived from the cellulose, and the contents of the plant cells are certainly liberated. The omasum has its mucous membrane raised into numerous laminae, and its sole function appears to be the filtering off of coarse particles from the mixture that is to enter the abomasum. The latter, alone of the four divisions, has a lining epithelium of secretory cells.

During the course of work on the physiology of the nematodes inhabiting the abomasum, it became necessary to obtain some idea of the limits of osmotic pressure reached by the abomasal contents, and it was decided to conduct a series of measurements on these contents. The freezing-point method was chosen, and the standard Beckmann apparatus used, following the precautions recommended by Nernst (1923). Such a method and such material did not allow accurate readings to the third place of decimals, and so the third place in the tables is given to the nearest 0.005.

The first series of measurements (Table I) were conducted on sheep, aged approximately 10–12 months, which were killed at a local abattoir. Previous to slaughter they had been starved for 12–16 hours, but with the exception of the last hour or hour and a half of this time they had received an ample water supply. The short period of starvation makes for little difference from the normal, since even after 7 days' starvation the fore-stomachs still contain food (Magee, 1932).

Table I.  $\Delta$  for the abomasal contents

No. 1. 0.555	No. 5. 0.565	No. 9. 0.580	No. 13. 0.600
„ 2. 0.555	„ 6. 0.570	„ 10. 0.590	„ 14. 0.605
„ 3. 0.560	„ 7. 0.570	„ 11. 0.595	„ 15. 0.610
„ 4. 0.560	„ 8. 0.570	„ 12. 0.595	

Considering the ante-mortem watering, these figures are sufficiently constant to be interesting, and possible explanations of the “constancy” were looked for. The most plausible explanation was found in the work of Trautmann (1933) who, on the basis of experiments involving the introduction of solutions into fistulae of the various compartments, reached the conclusion, contrary to current conceptions, that absorption took place from all four compartments but was less rapid from the abomasum than from the other three. This absorption would obviously tend to keep the osmotic pressure of the contents of the compartments identical with that of the blood, and so the values given in Table I should vary only between the limits found for  $\Delta$  for the blood of sheep. For the latter there are two values in the literature—0.619 quoted by Matthews (1925), and the average figure of 0.567 given by Trautmann *et al.* (1931): my own measurements, eleven in number, gave values ranging between 0.570 and 0.630, values which compare favourably with those of the abomasal contents.

The evidence so far given indicated the probable correctness of Trautmann's conclusions, but the relation between  $\Delta$  for the blood and  $\Delta$  for the abomasal contents was pursued further, and a series of measurements was made on the stomach contents and blood of three lambs, aged approximately 4 months, killed directly after removal from the pasturage. The results, upon which no comment need be given, are arranged in Table II.

Table II. Comparison of  $\Delta$  for blood and abomasal contents

No.	$\Delta$ for blood	$\Delta$ for corresponding abomasal contents
1	0.620	0.620
2	0.585	0.590
3	0.630	0.610

Trautmann's work extended over the four stomach compartments, and so, from the point of view of interest, a comparative series of measurements was conducted on the contents of the rumen, reticulum and abomasum, and on the blood of two 4-month-old lambs. The figures are given in Table III. Unfortunately, owing to the nature of its contents,

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which, consequent on its function are more or less solid, it is not possible to apply the freezing-point method to the omasum.

Table III. *Comparison of  $\Delta$  for the contents of the rumen, reticulum and abomasum, and for the blood*

No.	Blood	Rumen	Reticulum	Abomasum
1	0.625	0.625	0.625	0.620
2	0.630	0.630	0.630	0.630

#### SUMMARY

1. A comparison is made of  $\Delta$  for the blood of sheep with  $\Delta$  for the contents of the stomach compartments. It is suggested that the approximate identity of the two sets of values is best explained by the view that absorption takes place from the compartments.

2. A variation of 0.570–0.630 is recorded for  $\Delta$  for the blood of

The work was done in the Zoological Laboratory of University College, Cardiff, and I wish to thank Prof. W. M. Tattersall for his help.

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## THE DETERMINATION OF PHOSPHORUS IN SOILS

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IN the course of an investigation on soil phosphorus the writer was led to enquire into the methods used for its determination. Conventional methods have hitherto been adopted in order to determine the phosphoric acid soluble in hydrochloric acid. For many years the method commonly used in Great Britain was that of Hall<sup>(1)</sup> in which definite quantities of soil and hydrochloric acid (boiling-point  $110^{\circ}\text{C.}$ ) were heated for 48 hours in a loosely stoppered round-bottomed flask in a water-bath. Later (1929) the International Society of Soil Science<sup>(2)</sup> adopted the Van Bemmelen-Hissink method, whereby definite quantities of soil and hydrochloric acid (boiling-point  $110^{\circ}\text{C.}$ ) are boiled over an open flame for 1 hour in a flask fitted with a reflux condenser. This latter method of extraction was regarded as unnecessarily difficult in manipulation by the Analysis of Soils Sub-Committee of the Agricultural Education Association and a modification of it was provisionally adopted in 1931<sup>(3)</sup>. Instead of boiling the soil with acid (boiling-point  $110^{\circ}\text{C.}$ ) in a flask under reflux it was considered easier to carry out this operation in a tall beaker covered with a clock glass.

It has generally been supposed that the bulk, if not quite all, of the soil phosphorus is extracted by any one of these methods, but the quantities extracted are not identical and consequently the necessity arose for prescribing conventional methods in which proportion of soil to acid, and time of extraction, are stated.

### EXPERIMENTAL

With a view to getting some definite information on the effect of time of extraction, a number of soils was extracted with hydrochloric acid (boiling-point  $110^{\circ}\text{C.}$ ) for various times up to 48 hours, followed by a 48-hour extraction of the residue. In each case 10 gm. soil were digested with 200 c.c. hydrochloric acid (boiling-point  $110^{\circ}\text{C.}$ ) under a reflux condenser, as in the Van Bemmelen-Hissink method, and the usual

procedure was followed for the determination of  $P_2O_5$  in the acid extract, viz. evaporation of an aliquot portion to dryness, followed by gentle ignition combined with grinding and further ignition until a dry red powder was obtained. The residue was then boiled with 50 c.c. dilute  $H_2SO_4$  for 30 min., filtered, and the ammonium phospho-molybdate precipitated. The  $P_2O_5$  was calculated from the weight of blue-black precipitate obtained by gentle ignition. Preliminary experiments indicated the factor 0.038 as most suitable for conversion of the weight of blue-black residue to  $P_2O_5$ .

As the above technique was found to be laborious, time-consuming, and liable to error, especially during the evaporation and ignition stages, a more convenient method was sought. An adaptation of the method used by Richards and Godden(4) for biological material was tried and found satisfactory. The method, as finally adopted, consists in transferring an aliquot portion of the hydrochloric acid extract to a 500-c.c. Kjeldahl flask (pieces of porous tile are added to prevent bumping). 10 c.c. of concentrated sulphuric acid and 15 c.c. concentrated nitric acid are added and the mixture digested over a low flame until brown fumes cease to be evolved and the flask is full of white fumes. If necessary, more nitric acid is added and the digestion continued until the contents of the flask are quite white. When cold the solution is diluted with a small quantity of water and thoroughly shaken. It is then filtered into a 400-c.c. beaker, the residue thoroughly washed with hot water and the ammonium phospho-molybdate precipitated.

Results obtained by the two methods are given in Table I.

Table I. *Comparison of the results obtained by the ordinary method with those obtained by the  $H_2SO_4$ - $HNO_3$  method*

Soil	Period of extraction hours	$P_2O_5$ %	
		Ordinary method	$H_2SO_4$ - $HNO_3$ method
X	48	0.148	0.151
X	6	0.140	0.142
I	3	0.675	0.684
II	3	0.292	0.296
III	3	1.816	1.808

These results, being considered satisfactory, the sulphuric-nitric method was used for all subsequent work on hydrochloric acid extracts.

Particulars of soils used in this investigation are given in Table II.

The results, showing the effect of varying times of extraction with hydrochloric acid (boiling-point  $110^\circ C.$ ) are given in Table III.

Table II. *Particulars of soils used*

I	Penrhyn. Garden soil, Caernarvonshire: $\text{CaCO}_3$ present
II	Chalk soil, Berkshire: $\text{CaCO}_3$ present
III	Greensand subsoil, Berkshire: $\text{CaCO}_3$ present
IV	Shaly loam, Caernarvonshire: pH 5.5
V	Light loam, Aberdeenshire: pH 5.3
VI	Tea-garden soil, India: pH 5.5
VII	Loam, Glamorganshire: $\text{CaCO}_3$ present
VIII	Podsol, B-horizon, Caernarvonshire: pH 4.7
IX	Light loam, Glamorganshire: trace of $\text{CaCO}_3$ present
X	Light loam, Denbighshire: $\text{CaCO}_3$ present
XI	Humus light loam, Caernarvonshire: pH 5.0
XII	Light loam, Caernarvonshire: pH 4.8
XIII	Barmera, Australia: pH 7.8
XIV	Roseworthy, Australia: $\text{CaCO}_3$ present
XV	Tshernosem, Hungary: $\text{CaCO}_3$ present
XVI	Carrington silt loam, Iowa: pH 5.6
XVII	Wooster silt loam, Ohio: pH 5.9
XVIII	Sandy shell soil, Holland: $\text{CaCO}_3$ present
XIX	Greyish brown loam, East Africa: pH 6.3
XX	Red loam, East Africa: pH 5.1

Table III. *Percentage  $\text{P}_2\text{O}_5$  extracted by hydrochloric acid*  
(B.P. 110° C.)

Soil	pH	Duration of extraction in hr.								Residue after 48 hr. re-ex- tracted for 48 hr.
		1	2	3	6	12	18	24	48	
I	Carb.	0.692	0.693	0.680	0.695	—	0.692	—	0.697	0.005
II	Carb.	0.297	0.299	0.294	0.297	0.312	—	0.304	0.297	0.005
III	Carb.	—	—	1.812	—	1.778	—	—	1.748	0.021
IV	5.5	—	0.365	—	0.365	—	0.369	—	0.372	0.003
V	5.28	0.433	—	—	0.450	—	—	0.459	0.489	—
VI	5.52	—	0.127	—	0.133	—	0.132	—	0.132	—
VII	Carb.	—	0.121	—	0.124	—	—	0.128	0.131	Nil
VIII	4.69	—	0.318	—	0.316	—	0.315	—	0.322	—
IX	Carb.	—	0.259	—	0.262	—	0.262	—	0.265	0.003
X	Carb.	—	0.137	—	0.141	0.141	—	0.143	0.150	—
XI	5.03	—	0.477	—	0.498	—	0.511	—	0.524	0.004
XII	4.78	—	—	0.320	0.325	—	—	0.351	0.387	0.004

These results show that a definite end-point in extraction is reached in the longest period, viz. 48 hours, with all soils examined. With many soils, particularly with most carbonate soils, the end-point is reached by a short extraction, whereas in the case of most acid soils, the extraction is only completed in the longer periods. It may be concluded, in general, that a 48-hour period of extraction is amply sufficient to extract all the acid-soluble  $\text{P}_2\text{O}_5$ , and that the amount thus extracted represents a definite category of soil phosphorus.

It was then decided to try the effect of a direct extraction of the soil

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itself using the  $\text{H}_2\text{SO}_4\text{-HNO}_3$  method. For this purpose 1–10 gm. soil, depending on the amount of  $\text{P}_2\text{O}_5$ , are digested with 15 c.c. concentrated sulphuric acid and 15 c.c. concentrated nitric acid (with more  $\text{HNO}_3$  if necessary), and the same procedure is then followed as previously described for the hydrochloric acid extract.

Mention should be made here that a method essentially similar to this was used at the Halle Station (5) for the estimation of total  $\text{P}_2\text{O}_5$  in soils, but, so far as the writer is aware, it has not come into general use.

The results are compared with those obtained by a 48-hour extraction with hydrochloric acid in Table IV. Each result is the mean of duplicate determinations.

Table IV

Soil	$\text{P}_2\text{O}_5$ %	
	HCl method	$\text{H}_2\text{SO}_4\text{-HNO}_3$ method
I	0.697	0.697
II	0.297	0.296
III	1.748	1.760
IV	0.372	0.366
V	0.489	0.491
VI	0.132	0.126
VII	0.131	0.128
VIII	0.322	0.331
IX	0.265	0.260
X	0.150	0.142
XI	0.524	0.513
XII	0.387	0.386
XIII	0.040	0.039
XIV	0.043	0.044
XV	0.191	0.185
XVI	0.104	0.104
XVII	0.214	0.210
XVIII	0.084	0.083
XIX	0.185	0.186
XX	0.161	0.169
Mean	0.3268	0.3258
(HCl = 100)	100	99.7

The close agreement between the mean results by the two methods shows that the convenient sulphuric-nitric acid method may be safely substituted for the more time-consuming and laborious hydrochloric acid method. It also suggests strongly that in both cases a definite category of soil phosphorus has been distinguished. The insignificant proportion of  $\text{P}_2\text{O}_5$  obtained by re-extraction of the hydrochloric acid residues (Table III) suggests that this category may be taken as the total  $\text{P}_2\text{O}_5$  of the soil. Any soil phosphorus surviving either method of extraction is probably either included within soil minerals, or is so insoluble as to play no part in the phosphorus cycle of the soil.

## SUMMARY

1. Phosphorus in hydrochloric acid extracts of soil may be conveniently determined by digestion with sulphuric and nitric acids.
2. By boiling soil with hydrochloric acid (B.P. 110° C.) for 48 hours under reflux an end-point of extraction is reached.
3. Digestion of soil with sulphuric and nitric acids gives results in agreement with results by 48 hours' hydrochloric acid extraction.
4. It is suggested that the phosphorus thus extracted represents a definite category of soil phosphorus, which may be taken as the total phosphorus present in the soil.
5. Details of the suggested method are given.

## APPENDIX

*Proposed method for total  $P_2O_5$  in soil using sulphuric and nitric acids*

1–10 gm. of air-dried soil which has passed a 2-mm. sieve (the amount taken should give a convenient weight of blue-black precipitate, viz. 0.15–0.25 gm., equivalent to 0.0057–0.0095 gm.  $P_2O_5$ ) are transferred to a 500-c.c. Kjeldahl flask and 10–15 c.c. concentrated sulphuric and 15 c.c. concentrated nitric acid added. The flask is heated over a small flame at first and finally over a larger flame until the flask is full of white fumes. In case of incomplete oxidation as indicated by a darkish colour of liquid and residue in flask, it is necessary to cool and add more nitric acid. Digestion is usually complete in  $\frac{3}{4}$ –1 $\frac{1}{4}$  hours, except in the case of soils with a high content of organic matter. The flask is allowed to cool and a small quantity of water added and thoroughly shaken. It is then filtered into a 400-c.c. beaker and the residue washed (filtrate and washings usually amount to about 150 c.c.). The filtrate is neutralised with 0.88 ammonia: 20 c.c. concentrated  $HNO_3$ , and 30 c.c. of 50 per cent. ammonium nitrate are then added. The liquid is heated to 70–75° C. and 60 c.c. of 3 per cent. ammonium molybdate added. The precipitate is allowed to stand overnight and then filtered through a weighed ignited Gooch crucible packed with asbestos and washed with 1 per cent. nitric acid. The yellow precipitate is gently ignited to a blue-black colour, cooled and weighed. The  $P_2O_5$  is then calculated by using the factor 0.038.



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# THE VALUE OF ARTIFICIALLY DRIED GRASS, SILAGE MADE WITH ADDED MOLASSES AND A.I.V. FODDER IN THE DIET OF THE DAIRY COW AND THEIR EFFECT ON THE QUALITY OF THE MILK, WITH SPECIAL REFERENCE TO THE VALUE OF THE NON-PROTEIN NITROGEN

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## INTRODUCTION

IN a recent paper<sup>(1)</sup> it was shown in an experiment of the change-over type that artificially dried grass was as effective for milk production as a ration of concentrates containing an equivalent amount of starch equivalent and protein equivalent. This type of experiment with its relatively short periods is not absolutely satisfactory, and it was decided to repeat the test over a longer experimental period, applying the principle of covariance to test the results as suggested by Bartlett<sup>(2)</sup>.

The question of the feeding value of the non-protein nitrogen fraction of the nitrogenous compounds in certain foodstuffs has long been debated and is of particular importance, in view of the interest which has recently been aroused in the making of silage of high crude protein content from grassland herbage.

It is not possible to review here the extensive, and somewhat conflicting, literature dealing with the role of non-protein nitrogenous compounds in the diet of the cow. It can, however, be stated that experiments have been carried out to test the efficacy of urea, asparagine, ammonium acetate and ammonium bicarbonate for cows and goats in milk, which indicate that these compounds can be of value for milk production. Ehrenberg<sup>(3)</sup> and his co-workers at Breslau and Kirsch at Königsberg<sup>(4)</sup> are at present investigating this problem.

Kirsch and Jantzon<sup>(5)</sup> have also shown that the non-protein nitrogenous compounds produced from protein during silage making are a good source of nitrogen for ruminants. When dairy cows were fed either clover hay containing 11.57 per cent. crude protein and 2.28 per cent. of non-protein nitrogen compounds (non-protein N  $\times$  6.25) or clover silage containing 8.37 per cent. crude protein and 5.95 per cent. non-protein

nitrogen compounds, there was little difference in milk production and fat content of the milk. The nitrogen balance of the animals was positive in all cases.

Wright and his co-workers(6) have also shown that the biological value for milk production of the nitrogen of silage made with and without added molasses or mineral acids is high, and does not differ appreciably from that of the nitrogen of fresh grass, which is of a high order.

Edin, Berglund and Andersson(7) tacitly assume that the non-protein nitrogenous compounds formed during the silage-making process have a feeding value equal to that of the original "true" protein in the fodder. They quote a figure for digestible "true" protein and also a digestible crude protein equivalent to original "true" protein and add a footnote saying, "which of these two figures most adequately expresses the protein value of the silage depends on the quality of the silage itself. The more the ammonia set free and the butyric acid formed, the more advisable it is to apply the lower figure."

An experiment was designed, therefore, to examine these points, in particular the nutritive value of artificially dried grass and the nutritive value of silage containing extra non-protein nitrogen.

Two silages were used, one prepared by the A.I.V. process to limit protein breakdown as far as possible, and the other prepared by the addition of molasses, a method which usually entails a fair degree of protein breakdown.

In order that small differences in nutritive value might be apparent, the rations of the animals were reduced to a low level, particularly in regard to the protein content, thus ensuring that the animals made full use of the nutrients in the materials tested.

#### DETAILS OF THE EXPERIMENT

Adopting the method of experimentation suggested by Bartlett(2) the experiment consisted of a preliminary period of 3 weeks during which all the cows were on the control ration, which was that fed to the main herd and consisted of hay, roots and concentrates. The groups were thereafter changed over gradually to their experimental rations and remained on these rations for a period of 17 weeks.

The treatments applied during the experimental periods were:

- (1) Ordinary winter rations of roots, hay and concentrates.
- (2) Artificially dried grass.
- (3) Low temperature silage made with added molasses.
- (4) A.I.V. fodder.

Twenty Shorthorn cows were selected for the experiment, and were divided into five groups or blocks of four cows each, according to stage of lactation, milk yield and age. The cows of each group were then distributed at random over the four treatments, thus giving five replicates.

The details of the cows in the various groups and their allocations to the different treatments are given in Table I. The cows stood in the

Table I. *Details of cows used in the experiment*

Group	Name	Calving date	Age	Treatment
A	Mollie 2	26. vi. 34	5 calves	1
A	Rhoda	20. vi. 34	5 "	2
A	Queenie	25. vii. 34	3 "	3
A	Connie	25. vii. 34	2 "	4
B	Betty	12. ix. 34	4 "	3
B	Martha	11. ix. 34	3 "	2
B	Kathy 3	31. x. 34	5 "	4
B	Cherry	13. x. 34	1 calf	1
C	Derby	14. viii. 34	7 calves	2
C	Edna	18. viii. 34	5 "	3
C	Norah 2	10. viii. 34	2 "	1
C	Snowdrop	5. ix. 34	2	
D	Violet	—	1 calf	2
D	Ivy	—	1 "	3
D	Eileen	7. xi. 34	2 calves	4
D	Ladybird	7. xi. 34	1 calf	1
E	Gladys	19. xi. 34	2 calves	3
E	Princess	—	3 "	2
E	Mabel	—	3 "	1
E	Annette	—	3 "	4

cowshed in the same order as they are listed above, so as to exclude any possibility of their position affecting the results. Partitions were erected in the manger between the cows, so that it was impossible for any one animal to reach the ration of another. No trouble was experienced from the fact that adjacent animals were receiving different rations, and they all ate their rations well throughout the course of the experiment.

The pre-experimental period started on December 2, 1934 and finished on December 23. On the latter date, the change-over to the experimental rations was commenced, and extended over the period December 23–January 6, 1935. The cows then continued on the experimental rations until April 21, after which they were turned out to grass. During the course of the experiment the cows were allowed out to exercise, but care was taken that they should not have access to grass.

Unfortunately two cows in Group A, which contained the earliest calved animals, began to fall off in their yield after 8 weeks, so this group was regarded as ended after February 17. From this date to the end of the experiment there were only four groups under test.

The cow Derby, on dried grass, in Group C was suspected to be tuberculous and lost condition in the second half of the experiment. The effect on the milk yield was not sufficient, however, to warrant omitting her; it can be regarded as part of the experimental error.

The cow Gladys, on molassed silage (Group E), developed a hard quarter during the week ending January 13, and her milk yield fell very heavily and the original level was never resumed. In considering the results of the treatment, this cow has been omitted.

### *Composition of the foodstuffs used*

The foodstuffs used during the course of the trial were sampled frequently. The hay, which was baled, was sampled daily, as was the artificially dried grass. The dry-matter content of the daily samples was determined and a weekly composite sample was made up for analysis. In order to avoid any marked variation in the composition of the artificially dried grass, the crude-protein content of every separate bale was determined before the experiment began, and the bales were used as required. The hay used up to January 20 was of poor quality, but thereafter a better quality was used.

The molassed silage and A.I.V. fodder were sampled every second day and weekly samples made up as with the other roughages. The two silages were also sampled weekly by means of a boring, so that the ration could be adjusted at the beginning of the following week. The concentrate mixture used was sampled weekly and analysed. By the kindness of the manufacturers, the detailed make-up of the cube used was divulged, so

Table II. *Average composition of the foodstuffs used in the trial*  
(stated as percentages of the dry matter)

	Artifi- cially dried grass	Silage made with added molasses	A.I.V. fodder	Hay	Concen- trate mixture <sup>a</sup>	Man- golds	Oat straw
Ether extract	3.10	4.82	5.39	1.53	7.98	0.27	1.74
Crude fibre	24.18	20.49	19.55	31.72	8.18	6.96	40.94
Crude protein	18.33	17.64	21.01	10.03	22.07	10.01	2.82
Ash	9.05	17.77	15.65	7.39	6.47	12.63	7.39
N-free extractives	45.34	39.28	38.40	49.33	55.30	70.13	47.11
Organic matter	90.95	82.23	84.35	92.61	93.53	87.37	92.61
"True" protein	16.41	9.88	13.40	8.17	20.60	3.74	2.44
Calcium (CaO)	1.04	0.96	0.76	0.91	0.57	0.24	0.62
Phosphorus (P <sub>2</sub> O <sub>5</sub> )	0.80	0.73	0.85	0.61	1.83	0.67	0.21
Ratio "True" protein Crude protein	0.90	0.56	0.64	0.80	0.93	0.37	0.87
Moisture in fresh material	13.88	76.89	77.21	14.55	11.00	88.80	14.00

that it was possible to calculate the nutritive value with a fair degree of accuracy.

The average composition of the foodstuffs used in the trial is given in Table II.

*Artificially dried grass.* No grass was dried at Jealott's Hill in 1934 owing to the drought, and therefore, for the purposes of this trial, we purchased a quantity of grass which had been dried on a band drier. The product was baled and was of good colour and high quality. The dry-matter content of the daily samples was determined and a weighted weekly composite sample was made up and its crude-protein content determined. This figure, supplemented by digestibility coefficients, was used to check the ration fed during the week, the bales having been previously selected on the basis of the known protein content of each bale. From these weekly composite samples 3-weekly composite samples were made up for complete analysis. There were six 3-weekly samples, the figures in Table II being the average of all.

The average crude-protein content of the artificially dried grass was reasonably high and the fibre content relatively low. A noticeable point is the high proportion of "true" protein, fresh grass usually containing 80-85 per cent. of the crude protein in this form. The colour of the dried product, which contained little herbage other than grass, was good, and the average of thirty-six determinations of the carotene content gave a value of 31.3 mg. per cent. of the dry matter with a range of 12.1-48.8 mg. There was a positive correlation between the carotene and the total nitrogen content of the thirty-six samples on which both these determinations were made. The value of  $r$  ( $n=36$ ) was 0.8266, which is highly significant, the value for  $P=0.01$  (odds of 100 to 1) being  $r=0.4243$ .

*Silage made with added molasses.* The silage selected for the experiment was made at Jealott's Hill in October 1934 from grassland herbage consisting of grasses with an admixture of clover. It did not turn out to be quite so rich in crude protein as had been expected, though as will be seen from Table II it averaged about 18 per cent. of the dry matter over the whole experimental period. The silage was made in a metal tower silo some 16 ft. high, the grass being filled in with an ordinary hay elevator and not cut up. Layers of 4 in. depth were filled in at a time, and sprinkled with a solution of molasses, suitably diluted, such that about  $\frac{3}{4}$  lb. of molasses were added per 100 lb. of green grass (16 lb. dissolved in twice the volume of water were added per ton of grass). A layer of 4½ ft. was built and allowed to heat for 1 day during which the temperature rose to between 80 and 100° F. A second layer was then added and the

filling continued on alternate days till some 20 tons of fresh grass had been filled in, after a period of 11 days. Concrete weights were then placed on the top and the silo covered. The silage turned out extremely well, and had a very pleasant smell and was a yellow-green colour. The first foot of the silage was rather darker in colour, and was fed to store cattle. The silage was sampled every second day, when the rations for the cows were weighed out. The dry matter of the silage was determined on these samples and 2 days' rations were weighed out at the same time. The dry-matter samples were bulked weekly for nitrogen determination, and a weighted mean sample was made up every 3 weeks for complete analysis. The average of the six samples thus obtained is given in Table II.

It is obvious that the sample was of good quality. The ash content was somewhat high, more especially in the early samples, this being due to soil which is picked up with the grass at this season of the year. The ether extract was high as might be expected, since it also includes any organic acids formed during the fermentation process. The fibre content was low and the calcium and phosphorus were relatively high. The ratio of "true" protein to crude protein shows that a fairly extensive breakdown of the protein had occurred. This will be referred to again later. The moisture content was normal. The silage was extremely good and, as will be seen later, the grass had undergone a rapid and extensive lactic fermentation—the chief point in making good silage.

*A.I.V. fodder.* This is the name given to silage made with added mineral acid according to the process introduced by A. I. Virtanen<sup>(8)</sup> in which the mass is rapidly brought to a pH of between 3.0 and 4.0 by the addition of the requisite amount of a dilute solution of mineral acids, usually a mixture of hydrochloric and sulphuric<sup>(9)</sup>. The fodder was made at the same time as the silage with added molasses, but from the herbage of another field. The grass used for A.I.V. fodder was of higher protein content than that made into molassed silage.

The grass for A.I.V. fodder was filled into a metal tower 24 ft. high, the hay loader being used for the purpose. Layers of 4–5 in. were filled in at a time and sprayed with the dilute acid solution (2*N* strength) at the rate of 15 gallons per ton of fresh material. As in the molassed silage, the herbage was left uncut and was trampled down thoroughly during filling. The silo was suitably drained. Filling was continuous and lasted 8 days, the material being weighted overnight with planks and movable weights. The silo held 29 tons and the uppermost layer was covered over with old sacks and weighted heavily with movable weights, mostly concrete posts. The top layer of the fodder was discarded when the silo was opened and

was fed to store stock. Below this the fodder was a yellowish green colour, slightly more yellow than the molassed silage, had a pleasant smell and was eaten readily by the stock. The average analysis of the six 3-weekly composite samples is given in Table II.

The ether extract value is tolerably high, showing that there had been some organic acid formation. The crude-protein content was high, and the proportion of "true" protein somewhat higher on the average than was the case with the molassed silage. This is as might be expected from Virtanen's claims. The ash content was fairly high again, due to adherent soil put into the silo with the grass. The calcium content was slightly lower than that of the ordinary silage, but no direct comparisons are possible. The dry-matter content of the fodder was similar to that of the molassed silage.

The quality of the silage and A.I.V. fodder cannot be judged accurately from the above figures. It is necessary to examine the acidity of the material and measure the quantities of volatile bases and acids which are contained. The pH of the silage was determined weekly in the juice extracted by means of an ordinary tincture press and using a quinhydrone electrode. The total acidity, volatile acids, amino acids and volatile bases

Table III. *The total acidity, pH value, volatile base, volatile acid and amino acid contents of the molassed silage and A.I.V. fodder (stated as percentages on the fresh fodder)*

Molassed silage						
Date	pH	Total acidity c.c. N/10	Volatile bases*	Amino acids*	Volatile acids % acetic	Lactic acid (by difference)
Jan. 3, 9 and 16	3.94	415	0.32	1.05	0.52	1.87
Jan. 23, 30 and Feb. 6	3.92	450	0.30	1.18	0.45	2.16
Feb. 13, 20 and 26	3.80	408	0.23	0.84	0.56	1.97
Mar. 6, 13 and 20	4.21	459	0.47	1.19	0.65	1.94
Mar. 27, Apr. 3 and 10	3.88	410	0.23	0.75	0.42	2.29

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A.I.V. fodder						
Date	pH	Total acidity c.c. N/10	Volatile bases*	Amino acids*	Volatile acids % acetic	Residual acidity equivalent to lactic acid
Jan. 3, 9 and 16	3.67	335	0.19	0.78	0.31	1.76
Jan. 23, 30 and Feb. 6	3.79	366	0.20	0.69	0.36	2.04
Feb. 13, 20 and 26	3.64	379	0.20	1.03	0.30	1.90
Mar. 6, 13 and 20	3.81	276	0.12	0.58	0.20	1.59
Mar. 27, Apr. 3 and 10	3.73	388	0.25	0.89	0.32	2.10

\* The volatile bases and amino acids are calculated as crude protein ( $N \times 6.25$ ) from the titration values.



were determined on the fresh silage once per week by the method of Foreman (10) using Woodman's modification (11). The figures are given in Table III as 3-weekly averages.

Examination of the pH values shows that the acidity of the A.I.V. fodder lay between the values pH 3.0 and 4.0 on all occasions, except during the second week in January, when it was 4.04. Over the whole period the average pH value was 3.73. The silage made with added molasses was, however, also very satisfactory in this regard. A layer of rather poorer quality was encountered during March when the average value was pH 4.18. For the whole experimental period the average pH was 3.95. The addition of molasses resulted in the formation of sufficient lactic acid to increase the acidity rapidly to a level at which undesirable fermentations were kept in check. The total acidity as determined by titration of the extract from the silage, to which alcohol has been added as in the Foreman method, shows, as might be expected, higher values for the silage made with added molasses than for the A.I.V. fodder. The volatile bases, calculated as ammonia, are some 63 per cent. higher in the molassed silage than in the A.I.V. fodder, but even then the figures in the former are not high. The poorer quality molassed silage obtained in March, when the volatile base figure rose to its highest value, increased the average for the experimental period. The two sets of figures uphold Virtanen's (8) claims that the breakdown of the protein is reduced by the addition of mineral acids. This is shown too by the somewhat larger amounts of amino acids present in the molassed silage (equal to 1.00 per cent. crude protein) as compared with the A.I.V. fodder (equal to 0.79 per cent. crude protein). The figures for amino acids are relative and are based on the titration of the alkalinity developed in the alcoholic silage extract after distilling off the volatile bases, which value is called amino acids by Foreman. The titration values have been calculated in terms of crude protein. The figures for total volatile acids show also that these are lower in the A.I.V. fodder. They have been calculated in terms of percentages of acetic acid in the fresh mass, since this acid formed the major part of the volatile acids. The A.I.V. fodder was free from butyric acid throughout. The molassed silage was also free from butyric acid except for the samples of March 13 and 20, which contained 0.58 per cent. total acetic and 0.19 per cent. total butyric acid and 0.54 per cent. total acetic and 0.28 per cent. total butyric acid respectively. The greater amounts of volatile acids, amino acids and lactic acid in the silage made with added molasses are, of course, responsible for the higher values for total acidity in this material. In the case of the molassed silage it is

possible to take the difference figure between the total acidity value and the sum of the amino acid and volatile acids and call it lactic acid, a method of calculation which is common and will give figures somewhere near the truth in ordinary silage. The figures show that there had been a strong lactic acid fermentation in the molassed silage, the percentage in the fresh silage averaging 2.05 per 100 gm. of fresh silage throughout the experimental period. The residual acidity in the A.I.V. fodder, only part of which is due to lactic acid, has also been calculated as if it were all lactic acid, for comparative purposes. This value, calculated as lactic acid, was 1.88 per cent. per 100 gm. of fresh A.I.V. fodder.

The greater dissociation of the added mineral acids is responsible for the greater acidity of the A.I.V. fodder as measured by the pH value, whereas the total acidity was greater in the silage with added molasses.

In conclusion it may be said that though the A.I.V. fodder had turned out an excellent fodder, the silage made with molasses was not far behind it and would also be classed as an excellent silage.

*Carotene content of the silage.* The carotene content of the molassed silage and A.I.V. fodder was determined on three occasions during the course of the experimental period, and showed some considerable variation.

Table IV. *Carotene contents of molassed silage and A.I.V. fodder*  
(mg. per cent. of dry matter)

	Molassed silage	A.I.V. fodder
14. ii. 35	44.3	62.0
28. ii. 35	37.3	57.1
14. iii. 35	67.9	52.2
Average	49.8	57.1

The carotene content of the two silages was high, the A.I.V. fodder particularly so, and both are richer than the artificially dried grass in this respect. The A.I.V. fodder contained a very high amount of carotene throughout. The carotene in the molassed silage is, however, adequate if 30 lb. per head of silage were fed daily, as will be seen later.

*Hay.* It has already been mentioned that the hay was changed on January 20. The crude-protein content in the first two periods was only 7.01 per cent., whereas in the last four periods it was about 11.0 per cent. The first hay was a meadow hay, and would be classed as a poor hay on the basis of figures in the literature (12), though the farmer would have called it an average sample. The second hay was a "seeds" hay containing a fair proportion of clover and approximated in composition to values

usually ascribed to such material. The carotene content of the hay was low; the first sample of meadow hay contained 0.44 mg. per cent. of the dry matter, whilst the "seeds" hay contained 0.72 mg. per cent.

*Other foodstuffs.* The other foodstuffs used were the concentrate mixture, kale, mangolds, a little oat straw and a smaller amount of crushed oats.

The concentrate mixture was made up principally of decorticated ground-nut cake and rice meal ( $47\frac{1}{2}$  per cent.) with a smaller amount of decorticated cotton cake, maize meal, malt culms, bran, "weatings", whole wheat, bean meal and treacle. The mangolds, oat straw and oats were grown on the farm. Though weekly samples were made up, it is only necessary to quote the average analyses for the whole experimental period for the concentrates, mangolds and oat straw (Table II).

The marrow-stem kale was not analysed, as it was fed only during the pre-experimental period, nor were the oats which were only fed during the last 2 weeks of the experimental periods.

The analytical data are in line with figures usually given in the literature (12).

*Carotene content of other foodstuffs.* The concentrate mixture contained no carotene. The marrow-stem kale would be rich in carotene, but was only fed in the preliminary period.

Mangolds are almost devoid of carotene. The value for the oat straw and oats can also be ignored.

#### *Digestibility of the constituents of the ration*

In view of the knowledge of the composition of the concentrate mixture, it was decided not to determine the digestibility of the mixture but to calculate the nutritive value from average digestibility figures in the literature applied to the actual analysis of the material.

Average values were similarly taken for the mangolds, and since only small amounts of oat straw were fed, the usual digestibility coefficients for the latter foodstuffs were applied to the values determined on analysis.

Digestibility trials were started on the hay, artificially dried grass, molassed silage and A.I.V. fodder as soon as possible. Until the results of the digestibility trials were completed, the equivalence of the rations fed to the cows on the different treatment was based on average figures for similar materials in so far as starch equivalent was concerned, and the protein was based on the digestible nitrogen as determined by the pepsin-HCl method of Wedemeyer, given by Wiegner (13). The figures used are given in Table V.

Table V. *Average digestibility of the total nitrogen of the hay, artificially dried grass and concentrates used in the early stages of the experiment*

			%
Hay (3 samples)	...	...	79.2
Artificially dried grass (18 bales)	...	...	81.5
Concentrate mixture (2 samples)	...	...	91.1

The digestible crude protein in the silage was calculated on a basis of previous trials for similar material, a digestibility coefficient of 75 per cent. being assumed for the crude protein.

The digestibility of the molassed silage was checked again in February as the protein content was somewhat variable, and in March the digestibilities of the artificially dried grass, molassed silage and A.I.V. fodder were again checked by metabolism trials, two sheep being used at each test on each of the foodstuffs.

The coefficients obtained are given in Table VI.

Table VI. *Digestibility coefficients of the hay, artificially dried grass, molassed silage and A.I.V. fodder used in the experiment (stated as percentages)*

	Meadow hay	Seeds hay	Artificially dried grass			Molassed silage				A.I.V. fodder		
			Dec.	Mar.	Av.	Dec.	Jan.	Mar.	Av.	Dec.	Mar.	Av.
Dry matter	62.6	63.3	73.2	71.4	72.3	68.7	61.5	68.7	66.3	57.6	68.5	63.0
Organic matter	64.6	65.2	76.0	74.0	75.0	74.8	73.1	72.0	73.3	70.1	73.8	71.9
Ether extract	42.7	53.9	49.6	61.9	55.8	67.1	64.0	59.7	63.6	50.0	63.5	56.8
Fibre	64.8	65.0	78.8	78.2	78.5	79.3	68.9	73.7	74.0	77.7	81.7	79.7
N-free extractives	68.3	68.1	77.3	74.1	75.7	72.7	76.6	73.4	74.2	67.9	71.4	69.7
Crude protein	40.6	54.2	73.6	70.8	72.2	76.6	69.8	70.3	72.2	71.6	73.2	72.4
'True' protein	33.5	50.0	71.3	71.3	71.3	53.1	45.9	51.2	50.1	56.7	58.8	57.8

The digestibility of the hays was fairly high, the meadow hay, as might be expected, showing a lower digestibility of the protein, but the fibre was of surprisingly high digestibility. The coefficients for the artificially dried grass show it to be a product of high nutritive value, and there was good agreement between the two samples except in the case of the ether extract. This constituent, however, is low in amount and is the least accurate of the determinations. The agreement between the pairs of sheep was fairly good in all cases. The molassed silage and A.I.V. fodder were both of high digestibility. The "true" protein, as might be expected from the breakdown which takes place in silage making, shows a lower

digestibility than the artificially dried grass. This depression is more marked in the molassed silage with its greater breakdown, but is also clearly seen in the A.I.V. fodder.

*Nutrients in the different foodstuffs*

During the course of the experiment, the digestible nutrients, starch equivalent and protein equivalent of the various foodstuffs varied somewhat, with the exception of the concentrate mixture. It is impossible in the scope of this paper to give these variations, so the nutrients have been calculated by applying the average of the digestibility coefficient figures to the arithmetical average composition of the foodstuffs throughout the period. Where no digestibility trials were carried out, Kellner's digestibility coefficients (14) were used. The starch equivalent, protein equivalent, and digestible crude-protein values, calculated as above, are summarised in Table VII.

Table VII. *Nutrients contained in the foodstuffs used in the experiment (stated as percentages of the dry matter)*

	Starch equivalent	Protein equivalent	Digestible crude protein
Marrow-stem kale	65.2	6.27	7.79
Mangolds	69.3	4.34	7.00
Meadow hay	42.1	2.36	2.85
"Seeds" hay	40.7	5.16	5.89
Artificially dried grass	59.0	12.47	13.23
Silage made with added molasses	49.2	9.01	13.23
A.I.V. fodder	49.8	11.53	15.48
Oat straw	19.8	0.82	0.92
Crushed oats	68.7	8.77	9.24
Concentrate mixture	74.0	17.80	20.90

The figures for the marrow-stem kale are from digestibility trials carried out at Jealott's Hill (15), and for the oats from *Rations for Live-stock* (12).

*Rations fed*

The basis of the rationing was to supply for maintenance 6.0 lb. of starch equivalent and 0.6 lb. of protein equivalent per 1200 lb. live weight. In addition, it was decided to feed 2.25 lb. of starch equivalent and 0.5 lb. protein equivalent or its equivalent for each gallon of milk produced.

These standards are lower than those usually advocated in the feeding of the dairy cow (16). It was considered necessary to feed at a lower level than that usually advocated, since the standards in general use are

undoubtedly on the high side, in order to allow for differences in the biological value of the great variety of foodstuffs which may be used. Unless the plane of nutrition is lowered, the margin of safety in the everyday standards may mask any differences in the value of the foodstuffs under test.

During the preliminary period, the maintenance ration consisted on the average of 14 lb. of hay and 30 lb. of marrow-stem kale per head daily. For the production of milk  $3\frac{1}{2}$  lb. of the proprietary balanced dairy cube were fed per gallon, the amount being reduced to 3 lb. per gallon by December 23. In order to establish a basis of equivalence for the nitrogen fed, this ration was calculated in terms of digestible crude protein. After December 23, the kale was replaced by mangolds and 20 lb. were fed per head daily until January 10, after which the amount was increased to 40 lb. per head daily.

The rations fed after December 23 were gradually changed to the following:

*Treatment 1. Control winter diet.* Hay to make up, together with the roots, a maintenance ration supplying 6.0 lb. of starch equivalent and 0.6 lb. protein equivalent per 1200 lb. live weight. Cake to supply 2.25 lb. starch equivalent and 0.5 lb. digestible true protein per gallon of milk. This latter amount of digestible true protein was equivalent to just over 0.6 lb. of digestible crude protein.

*Treatment 2. Artificially dried grass.* After the preliminary period of 3 weeks, it was arranged that the largest possible amount of artificially dried grass should be fed. The cows were started at 15 lb. per head daily. Since, however, it was essential that the amount of nitrogen fed in the dried grass or in either of the silages should be equal, the amount was fixed by the animal with the lowest appetite. The smallest cow, a heifer, Ivy, on the molassed silage ration could not eat more than 35 lb. per day. In order to allow a margin, the amount of this silage was put at 30 lb. per head per day, though after the eighth week it was raised to 35 lb. per day, and a weight of dried grass containing an equivalent amount of digestible nitrogen was fed. The maintenance ration consisted of hay and roots adjusted to the individual weights, and any surplus requirement was made up with the concentrate mixture.

It will make it clearer if the rations fed are summarised, and this has been done in Table VIII. The rations are divided into three periods, the pre-experimental period, a first experimental period of 8 weeks when there were five groups of cows, and a final experimental period of 9 weeks when there were only four groups of cows under test.

Table VIII. *Rations fed during the preliminary and experimental periods (average ration fed per head per day in lb.)*

	Kale	Man-golds	Hay	Oat straw	Concentrates	Oats	Artificially dried grass	Silage with added molasses	A.I.V. fodder
Treatment 1. Control winter diet:									
Pre-experimental period	30	—	14	—	11.7	—	—	—	—
Experimental period 1	—	22.5	12.4	—	10.2	—	—	—	—
Experimental period 2	—	40.0	6.8	—	8.0	—	—	—	—
Treatment 2. Artificially dried grass:									
Pre-experimental period	30	—	14	—	10.3	—	—	—	—
Experimental period 1	—	22.5	12.7	—	2.5	—	9.3	—	—
Experimental period 2	—	40.0	8.0	—	0.4	—	7.8	—	—
Treatment 3. Silage made with added molasses:									
Pre-experimental period	30	—	14	—	11.7	—	—	—	—
Experimental period 1	—	22.5	12.3	—	4.5	—	—	27	—
Experimental period 2	—	40.0	6.5	1.3	1.0	0.2	—	34	—
Treatment 4. A.I.V. fodder:									
Pre-experimental period	30	—	14	—	11.5	—	—	—	—
Experimental period 1	—	22.5	11.8	—	4.2	—	—	—	31
Experimental period 2	—	40.0	6.7	1.1	1.1	0.6	—	—	27

The artificially dried grass was fed at the rate of 9.3 lb. per head daily during the first experimental period and 7.8 lb. in the second.

*Treatment 3. Silage made with added molasses.* After the preliminary period of 3 weeks, silage was introduced into the ration, and throughout the first experimental period 27 lb. were fed per head daily. The amount rose to an average of 34 lb. in the second experimental period as the crude protein content of the silage fell in the lower part of the silo. The maintenance ration, as with the other groups, consisted of hay and mangolds, and concentrates were fed to make up the ration to the total requirements. In the second experimental period the yield of one cow fell to such a level during the last few weeks that the hay had to be replaced by oat straw to keep the nitrogen intake at the correct level, and in the last 2 weeks crushed oats had to be fed instead of the concentrate mixture.

*Treatment 4. A.I.V. fodder.* In the first experimental period an average of 31 lb. of A.I.V. fodder was fed per head daily, and the ration was made up to the required level with mangolds, hay and concentrates. In the second experimental period an A.I.V. fodder of higher nitrogen content was encountered lower in the silo, and 27 lb. were fed daily per cow. Here again one cow had fallen to such a low level of yield towards the end of the experiment that it proved necessary to replace the hay by oat straw, and crushed oats had to be used during the last 2 weeks of the period to balance the rations. Both the molassed silage and A.I.V. fodder

were fed in amounts which provided the same amount of digestible crude protein as did the artificially dried grass and control rations.

The rations fed agreed well with the standards laid down for the experiment, as may be seen from Table IX. In calculating the figures, the

Table IX. *Nutrients fed compared with the standards decided upon (average per cow)*

Group	Starch equivalent				Increase (+) or decrease (-) over requirements %
	Actually fed lb.	Maintenance lb.	Production lb.	Total lb.	
1. Control	1420.6	618.8	750.5	1369.3	+3.7
2. Artificially dried grass	1358.6	654.5	629.1	1283.6	+5.8
3. Molassed silage	1360.8	630.7	666.1	1296.8	+4.9
4. A.I.V. fodder	1332.4	630.7	731.6	1362.3	-2.2

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Group	Digestible crude protein				Increase (+) or decrease (-) over requirements %
	Actually fed lb.	Maintenance lb.	Production lb.	Total lb.	
1. Control	278.6	70.2	206.8	277.0	-0.6
2. Artificially dried grass	227.9	76.2	173.3	249.5	-8.6
3. Molassed silage	259.2	71.4	183.6	255.0	+1.7
4. A.I.V. fodder	273.6	71.4	201.6	273.0	±0.0

four cows which completed the whole experimental period have been used for the treatments 1, 2 and 4, and Gladys, the cow which developed a hard quarter, has been omitted from treatment 3—silage made with added molasses—leaving three cows on this treatment.

The maintenance requirements have been calculated on the basis of the average weight of the cows in each group during the experimental period. The production requirements are average values calculated from the total milk yield.

The amounts of nutrients actually fed are calculated from the average weight of food fed per cow in each of the groups during the 17 weeks in question.

During the preliminary period the amount of starch equivalent fed was somewhat high. This was due to the fact that the kale and hay fed supplied more nutrients than were required for the level of maintenance decided upon for the remainder of the experiment.

After the first 3 weeks of the experimental period, during which the rations were being changed over, the level of starch equivalent was



nearer to that decided upon. As a result, however, the average allowance of starch equivalent per gallon of milk throughout the experimental period was slightly too high for treatment 1, still higher for treatments 2 and 3. For treatment 4, the allowance was slightly below the standard decided upon, due chiefly to an overestimate of the starch equivalent value of the A.I.V. fodder in March, just prior to the second digestibility trial.

The digestible crude protein fed during the preliminary period was up to the standard required.

In the experimental period there was a satisfactory agreement between the amounts of digestible crude protein fed and the amounts needed according to the standards set down for Groups 1, 3 and 4. The amount fed to Group 2 on the artificially dried grass ration was too low by 8.6 per cent. This is the largest difference which shows in any of the rations.

In view of the practical difficulties of such an experiment, the level of nutrient intake is satisfactory.

The percentages of the total intake of digestible crude protein supplied by the artificially dried grass, silage with added molasses and A.I.V. fodder are given in Table X.

Table X. *Digestible crude protein supplied by artificially dried grass and silage additions (average weight per cow)*

Treatment	Digestible crude protein in dried grass or silage lb.	As % of total digestible crude protein in ration
Artificially dried grass	0.98	51.0
Molassed silage	1.04	49.1
A.I.V. fodder	1.09	48.5

They have supplied on the average half of the total intake of digestible crude protein. In the second and third weeks the artificially dried grass supplied about three-quarters of the total intake. This was when 15 lb. of dried grass were being fed. When it was reduced to conform with the maximum appetite of the cow eating the lowest ration of silage it fell again.

The non-protein nitrogen formed 11.6, 63.9 and 51.0 per cent. of the total digestible nitrogen in the artificially dried grass, silage with added molasses and A.I.V. fodder respectively. This means that 5.9 per cent. of the total digestible nitrogen intake in the artificially dried grass group, 31.4 per cent. in Group 3 (the silage made with added molasses group), and 25.0 per cent. in Group 4 (the A.I.V. fodder group) was in the form of

non-protein nitrogenous compounds supplied by the foodstuffs in question. This is an appreciable amount, particularly in the ration of silage made with added molasses, more especially when it is realised that the intake of protein was reduced to a level well below that usually employed in the feeding of the dairy cow.

The basal ration contained mangolds which are also rich in non-protein nitrogen. Throughout the whole experimental period an average daily ration of 31.75 lb. of mangolds was fed per cow. This contained 0.22 lb. non-protein nitrogen stated as crude protein (*e.g.*  $N \times 6.25$ ) representing 9.4, 11.4, 10.4 and 9.8 per cent. of the total digestible crude protein in the rations of Groups 1, 2, 3 and 4 respectively. During the first 7 weeks, 20 lb. of mangolds were fed per head per day, and for the remaining 9 weeks 40 lb. per head, the average values for the first period being 5.3, 6.4, 5.7 and 5.2 per cent., and for the second 13.0, 16.3, 14.2 and 13.7 per cent. for the respective groups.

This raises the level of the total non-protein nitrogen fed from 20 up to as much as 44 per cent. of the digestible total nitrogen in the rations.

### *Milk yield*

The cows were all milked twice daily, and the weights of milk produced at every milking were recorded in the usual way. The individual total weekly yields can be examined by anyone interested<sup>(23)</sup>, and the average yields per treatment are summarised in Table XI.

Table XI. *Weekly average yield of cows under experiment*

Week ending	Treatment 1 Control	Treatment 2 Artificially dried grass	Treatment 3 Silage made with added molasses	Treatment 4 A.I.V. fodder
Pre-experi- mental { 9. xii. 34	242.5	215.7	236.0	257.0
16. xii. 34	244.0	214.5	239.9	259.2
23. xii. 34	242.3	213.5	239.6	258.5
30. xii. 34	237.3	202.4	231.2	251.3
6. i. 35	225.1	194.8	220.4	242.0
13. i. 35	218.1	190.6	197.6	220.6
20. i. 35	216.2	186.4	194.3	221.4
27. i. 35	211.2	179.7	191.3	221.5
3. ii. 35	209.4	177.9	187.6	213.2
10. ii. 35	206.2	171.2	173.7	201.9
17. ii. 35	203.3	169.7	180.9	202.8
24. ii. 35	196.7	168.4	171.3	192.2
3. iii. 35	189.7	165.0	165.8	183.2
10. iii. 35	188.4	159.0	161.2	177.5
17. iii. 35	188.1	153.1	153.8	171.1
24. iii. 35	183.9	149.1	150.8	161.7
31. iii. 35	179.0	135.6	149.9	156.7
7. iv. 35	167.2	134.7	144.0	148.2
14. iv. 35	162.9	126.3	143.6	142.1
21. iv. 35	153.0	132.1	143.2	144.3

For this purpose the four cows which completed the full period in treatments 1, 2 and 4 have been used and three cows in treatment 3, molassed silage. It has already been pointed out that one cow in this treatment—Gladys (Group E)—developed a hard quarter, and fell very heavily in milk yield, so was eliminated from the calculation of the results of the experiment.

In the statistical analysis of the results, for which thanks are due to Mr M. S. Bartlett, this cow was considered as missing. Since the cows of the first block did not remain in milk throughout the whole experiment, a statistical analysis is only possible for the whole five blocks for the first 8 weeks on the experimental ration. The remaining four blocks can be considered for the last 9 weeks of the experimental period and also for the whole 17 weeks. Rather less information is thus obtainable from the results than had been planned originally.

The milk yields are given in Table XII in the form required for analysis. The figures for each treatment, reading down the columns, are the average milk yield in lb. per week: (i) for the 3 weeks' preliminary control period, (ii) for the first 8 weeks of the experimental period, (iii) for the last 9 weeks, and (iv) for the whole 17 weeks of the experimental period.

Table XII. *Yield of milk in lb. per week of individual cows as averages of preliminary and experimental periods*

Block		A	B	C	D	E
Control	(i)	176	279	245	197	250
	(ii)	139	226	226	178	232
	(iii)	—	192	199	138	186
	(iv)	—	208	212	157	208
Artificially dried grass	(i)	206	223	224	179	232
	(ii)	174	190	185	171	191
	(iii)	—	156	146	143	143
	(iv)	—	172	164	156	166
Molassed silage	(i)	157	269	256	191	<b>249</b>
	(ii)	128	213	221	157	<b>216</b>
	(iii)	—	156	188	117	<b>159</b>
	(iv)	—	183	204	136	<b>188</b>
A.I.V. fodder	(i)	163	342	208	210	274
	(ii)	144	284	160	181	252
	(iii)	—	214	132	139	173
	(iv)	—	247	150	159	210

The figures in black type are estimates for the missing cow. It will be noticed that it is necessary, in order that there shall be no contribution to the regression from the missing cow when the final yields are adjusted by means of the initial yields, to estimate also this initial yield. Since the

set of cows differs for the analysis of the first 8 weeks and for the other two analyses, the values of 249 and 257 are obtained, the first applicable to the first analysis, and the second to the other two.

The test of the significance of treatment differences, block differences having been eliminated, is given in Table XIII. The first 8 weeks with five blocks, the second 9 weeks with four blocks and the whole 17 weeks with four blocks have been considered.

Table XIII. *Final yields  $y$  adjusted by initial yields  $x$ . Test of significance of treatment differences (block differences eliminated)*

	Degrees of freedom	$y_x^2$	Variance	Standard error
(1) First 8 weeks:				
Regression	1	7510	7510	—
Treatments	3	201	67.0	—
Error	10	1247	124.7	11.17 (5.76%)
Total	14	8958		
(2) Second 9 weeks:				
Regression	1	3259	3259	—
Treatments	3	1636	545.3	—
Error	7	1521	217.3	14.73 (9.13%)
Total	11	6416		
(3) Whole 17 weeks:				
Regression	1	4065	4065	—
Treatments	3	888	296.0	—
Error	7	1134	162.0	12.73 (6.98%)
Total	11	6087		

In the analysis for the last 9 weeks, the value of  $z$  for testing the effect of treatments is 0.460, while  $z$  ( $P=0.05$ ) is 0.735. This, though a larger value of  $z$  than that for either of the other two analyses, is thus not significant.

The estimated standard error per cow is given in the table and is given also as a percentage of the general mean for the period being analysed. It will be seen that the value for the whole period compares favourably with the value of 8.14 per cent. previously obtained (2) in discussing the application of this method of analysis to the results of dairy cow trials.

A summary of the adjusted mean yields per treatment is given below in Table XIV.

The difference between the control and the A.I.V. fodder ration just reaches the significant level in the second period, but no other differences are significant. It must be remembered that this is the greatest difference between the treatments that can be selected, and on the whole, as indicated by the  $z$  test, the results are not sufficient to justify a conclusion

that the treatments have had any effect. It would appear, therefore, that in so far as milk production is concerned the sample of artificially dried grass used produced a result which was to be expected from its composition and digestibility. This presumes that the degree to which the rations had been reduced did not allow of such an excess of nutrients that any difference in the value of the foodstuffs used was compensated thereby. The results show, furthermore, that for milk production the digestible crude protein of the foods tested, *e.g.* artificially dried grass, A.I.V. fodder and, more particularly, silage made with added molasses, is all utilised whether or not it is present in the form of non-protein compounds or as true protein. This holds good for a proportion of up to 30-40 per cent. of the nitrogen intake of the animals, the highest level at which the utilisation of non-protein nitrogen was tested in this experiment.

Table XIV. *Summary of mean yields of cows in lb. per week*

Period	Control	Artificially dried grass	Molassed silage	A.I.V. fodder	General mean	Standard error	Signi- ficant* difference
First 8 weeks (5 blocks)	197.7	194.4	188.9	194.7	193.9	5.00	15.8
Last 9 weeks (4 blocks)	176.5	165.7	152.4	150.6	161.3	7.37	24.6
17 weeks (4 blocks)	193.8	185.4	174.9	176.0	182.5	6.37	21.3

\* Significant difference figures ( $P=0.05$ ). These figures are not quite exact owing both to the missing cow and to the adjustments for initial yields. Although any amendment would, however, be slight, it should be noted that the figures given are always underestimates of the true level.

*Composition of the milk.* The milk was sampled at fortnightly intervals, individual samples being taken at the morning and evening milkings of one day. The fat content of the milk was determined by the Gerber method, and the solids-not-fat were calculated from the specific gravity in the usual way. The full analytical data can be examined by anyone interested (23).

*Percentage of butter-fat and solids-not-fat.* The actual percentages of butter-fat and solids-not-fat in the milk are given in Table XV as (i) averages for the preliminary period and (ii) averages of the experimental period, for the cows of the four groups which completed the whole experimental period. As with the milk yield, the figures in black type are estimates for the missing cow in place of Gladys, which was left out on account of a hard quarter. The figures are unweighted averages throughout, including no distinction between morning and afternoon

values. This omission does not affect the accuracy of the statistical examination.

Table XV. *Average percentage of butter-fat and solids-not-fat in the milk*

Block		Butter-fat %				Solids-not-fat %			
		B	C	D	E	B	C	D	E
Control	(i)	3.81	2.66	3.46	3.40	8.83	8.62	8.77	8.63
	(ii)	4.18	2.90	3.23	3.52	8.83	8.75	8.69	8.57
Artificially dried grass	(i)	3.14	2.94	3.38	3.24	8.73	7.42	8.61	8.31
	(ii)	3.51	3.35	3.60	3.46	8.98	7.40	8.71	8.40
Molassed silage	(i)	2.98	2.89	3.59	<b>3.35</b>	8.47	8.26	8.72	<b>8.50</b>
	(ii)	3.15	3.33	3.83	<b>3.50</b>	8.39	8.12	8.76	<b>8.37</b>
A.I.V. fodder	(i)	3.43	3.71	3.16	3.86	8.33	8.68	8.68	8.67
	(ii)	3.49	4.03	3.24	3.71	8.31	8.63	8.29	8.41

The solids-not-fat content of the milk produced by Derby (Group C, artificially dried grass) is extremely low. This is noticeable in both the preliminary and the experimental periods.

The adjusted unweighted averages of the percentages of butter-fat and solids-not-fat in the milk of the cows on the different treatments are summarised in Table XVI.

Table XVI. *Summary of adjusted unweighted average butter-fat and solids-not-fat contents of milk (per cent.)*

	Control	Artificially dried grass	Molassed silage	A.I.V. fodder	General mean	Standard error	Significant difference
Butter-fat	3.44	3.63	3.58	3.36	3.50	0.077	0.26
Solids-not-fat	8.47	8.67	8.44	8.32	8.48	0.059	0.20

There are no significant differences in percentage of butter-fat in the milk of the cows. It would appear as if the A.I.V. fodder treatment had produced a milk significantly lower in butter-fat content than that produced by the artificially dried grass, but as in the case of the milk yields it must be remembered (apart from the slight underestimate of the significant difference given) that this is the greatest difference possible in the table. The  $z$  test did not show the difference to be significant, so we must presume that there were no significant differences in butter-fat content of milk due to treatment.

The results for the examination of the solids-not-fat percentages do show a significant difference as judged by the  $z$  test ( $z=0.8087$ , while the  $P=0.05$  value of  $z$  is  $0.7347$ ). The use of artificially dried grass in the ration of the cows has produced a milk which is significantly higher than that of the group which received A.I.V. fodder.

The artificially dried grass treatment has been the most efficient as regards its effect on solids-not-fat content of milk, and the A.I.V. fodder the least.

It is worthy of note that one cow in the artificially dried grass group—Derby, Block C—gave extremely low values for solids-not-fat in both the preliminary period and the experimental period. This cow was suspected as tuberculous but was kept in the experiment. Had another cow with a more normal content of solids-not-fat in the milk been included, the mean value for this component of the milk would have been still higher though the significance of the results would remain unaltered.

The general mean of all the cows shows a value just below that of the presumptive legal standard of 8·5 per cent. of solids-not-fat in the milk, and all the treatments except the artificially dried grass are below the standard on average. This is not affected by the fact that the figures are unweighted averages, as it appears from an examination of the full analytical data that the solids-not-fat content of the morning samples is generally lower than that of the afternoon samples. Since the morning yield of milk was somewhat greater than that of the afternoon, the weighted value for solids-not-fat in the milk produced each day would be still lower than the arithmetic averages used in examining the data.

*Yield of butter-fat.* The daily yield of butter-fat can be worked out from the data for yield of milk and the fat percentage of the milk at each sampling date. This has been done for the cows of Groups B to E, Group A being excluded since they were only in the experiment for 8 weeks. The yields of butter-fat obtained by calculation were regarded as representative of the fortnight prior to the sampling date. The figures for the average daily yield of butter-fat are given in Table XVII, (i) for the preliminary period, and (ii) for the experimental period.

Table XVII. *Average daily yield of butter-fat in lb.*

	Block	B	C	D	E
Control	(i)	1·615	0·890	0·896	1·294
	(ii)	1·202	0·843	0·696	1·059
Artificially dried grass	(i)	1·081	0·928	0·895	1·150
	(ii)	0·833	0·769	0·790	0·766
Molassed silage	(i)	1·143	1·027	0·862	<b>1·306</b>
	(ii)	0·805	0·934	0·720	<b>0·911</b>
A.I.V. fodder	(i)	1·729	1·147	0·963	1·823
	(ii)	1·186	0·841	0·723	1·077

The figures in black type are those for the missing cow, as in the case of the milk yields.

The adjusted mean yields of butter-fat of the different treatments are given in Table XVIII as lb. of butter-fat per day.

Table XVIII. *Summary of adjusted mean daily yields of butter-fat (lb./cow)*

Control	Artificially dried grass	Molassed silage	A.I.V. fodder	General mean	Standard error	Significant difference
0.949	0.888	0.897	0.805	0.885	0.0648	0.153

There are no significant differences due to treatment, which is in agreement with the findings for the milk yield and the figures for the butter-fat content of the milk.

*Effect on colour of the milk.* This aspect of the experiment was of secondary importance. The yellow colour of the milk, as is well known, is due to the presence of carotene in the butter-fat. The carotene content of the bulked milk of the cows of each treatment was measured colorimetrically in a Lovibond tintometer, the curve put forward by Ferguson (17) being used to read off the amount of carotene.

The carotene contents of the different foodstuffs have already been considered. The ration fed in treatment 1 was low in carotene, whilst the use of artificially dried grass, molassed silage and A.I.V. fodder raised the carotene intake appreciably. It is not possible to give any accurate figures for the carotene content of the rations fed, but an approximation can be obtained by an application of the average carotene contents to the average weights of the different fodders fed. This gives intakes of carotene of 889, 1362 and 1453 mg. in the artificially dried grass, molassed silage and A.I.V. fodder fed in the respective rations. The amount of carotene in the control diet was negligible, since both hay and concentrates showed very low values for this constituent. The carotene contents of the butter-fat in the different treatments are given in Table XIX.

Table XIX. *Carotene content of butter-fat (stated as mg. per cent.)*

	12. xii.	27. xii.		10. i.	7. ii.	21. ii.	7. iii.	21. iii.	4. iv.	16. iv.	Av. 10. i.- 4. iv.
Sampled	1934	1934	Av.	1935	1935	1935	1935	1935	1935	1935	1935
Control	0.38	0.32	0.35	0.32	0.33	0.28	0.22	0.19	0.21	0.25	0.26
Artificially dried grass	0.73	0.66	0.70	0.99	0.67	0.80	0.96	0.92	0.78	0.82	0.85
Molassed silage	0.49	0.52	0.51	0.68	0.76	0.64	0.64	0.80	0.56	0.55	0.66
A.I.V. fodder	0.43	0.44	0.44	0.46	0.46	0.57	0.58	0.39	0.43	0.43	0.47

There was a rather wide difference between the groups at the first sampling on December 12.



The figures for the carotene content of the butter-fat during the preliminary period are fairly high. Marrow-stem kale was being fed, and the cows had only recently been taken off grass, and this probably accounts for the high values.

This was confirmed by the figures on December 27, only 4 days after the change-over was started and when the experimental ration could not have affected the colour and carotene content of the milk. The average of the experimental period values shows that the colour of the control milk has fallen to a marked degree. The artificially dried grass shows an average increase of 0.16 mg. during the experimental period and the molassed silage a like quantity. The increase on the A.I.V. fodder ration was only 0.03 mg. per cent., but no falling off in butter colour was noted. It is impossible to say if this lower efficiency is significant, but from past experience<sup>(18)</sup> it is most likely that the result is due to individualism in the latter stages of the trial, particularly since the increase in carotene on March 7 was 0.14 mg. per cent. All three additions to the diet have raised or kept the carotene content at a level similar to that obtained as an average of the grazing season in the butter-fat of cows on a diet of pasture herbage.

#### *Weights of cows*

The cows were weighed on three consecutive days at the beginning of the experimental period (December 19, 20 and 21) and again at the end (April 16, 17 and 18). The cows, as a whole, tended to show a loss of weight during the experimental period. To examine how far this might be due to particular treatments, an analysis was made of the weights of the fifteen cows remaining in the experiment for the whole period, corresponding exactly to the method of analysis of the milk yields.

Table XX. *Live weights of cows at the beginning of the experimental period and losses or gains in weight during the period*

Block		B	C	D	E
Control	(i)	103	107	95	94
	(ii)	- 33	+ 33	+ 16	+ 9
Artificially dried grass	(i)	112	127	82	112
	(ii)	- 20	- 130	+ 46	- 78
Molassed silage	(i)	108	113	85	103
	(ii)	- 61	- 72	+ 4	- 70
A.I.V. fodder	(i)	119	101	85	108
	(ii)	- 48	- 32	- 68	- 90

The figures in Table XX give (i) the initial weight (average of 3 days) in 10-lb. units, and (ii) the differences between the final weight (of

average 3 days) and the initial weight in 1-lb. units. A negative sign denotes a loss in weight.

The figures in black type represent the figures for the missing cow. Since any necessary adjustment is to be made for initial weights, the final results would be the same whether changes in weight or final weights themselves are considered. It is of interest, however, first to find the changes in weight as shown above, since it is then simpler to see whether these *changes* are correlated at all with initial weights.

Table XXI. *Test of significance of adjusted treatment differences (blocks eliminated)*

	Degrees of freedom	$y_w^2$	Variance	Standard error
Regression	1	6769	6769	—
Treatments	3	7283	2428	—
Error	7	10900	1557	39.46
Total	11	24952	—	—

The regression square is reasonably large. Though not significant ( $z=0.735$ , while  $Z (P=0.05)=0.861$ ), it suggests what might be expected—that the heavier cows tend to lose weight more readily than the lighter cows, which are still liable to gain in weight. The treatment variance is obviously not significant. A summary of the adjusted losses in weight is given in Table XXII.

Table XXII. *Change in weight in lb. per cow for the 17 weeks*

Control	Artificially dried grass	Molassed silage	A.I.V. fodder	General mean	Standard error	Significant difference
-5.6	-29.6	-53.4	-59.9	-37.1	19.73	66.0

The differences in change of weight are not significant, but there is an indication that the cows in the two silage treatments have lost more weight than the control, the difference not being so marked in the case of the artificially dried grass.

## DISCUSSION

The method of experimentation has proved eminently satisfactory and capable of practical application. The long experimental period allows the ration to exert its full effect, a point of particular importance where such points as the effect of the diet on the quality of milk and the value of non-protein nitrogen are concerned, since the effects are cumulative and may not show up where the experimental periods are relatively short.

The experiment has confirmed the finding that was obtained in the

previous short period, change-over, experiment (1), namely that artificially dried grass has replaced an equal weight of nutrients in the form of concentrated foodstuffs without significantly affecting the yield of milk, butter-fat percentage or live weights of the cows.

There is, however, an extremely important indication in the results for the solids-not-fat content of the milk. The diet of artificially dried grass, despite the presence of one cow which consistently produced a milk low in solids-not-fat, has produced a milk richer in solids-not-fat than that obtained when A.I.V. fodder was fed. Although the general mean of the solids-not-fat values was below the accepted standard, the dried grass treatment is the only one to exceed it. In view of the present widespread prevalence of low solids-not-fat during the winter in the milk of cows<sup>(19)</sup> and the correction of this when cows are turned out to grass, this is a point which is deserving of fuller investigation. The dried grass used was of good quality and colour, and it would be interesting to see whether this indication is justified by results obtained when feeding still larger amounts of good quality artificially dried grass. An indication of an increased content of calcium in the milk of cows on a diet of artificially dried grass has been obtained in a previous experiment<sup>(20)</sup>. The whole question calls for more detailed examination of the milk.

The starch equivalent and protein equivalent values calculated for the composition and digestibility of artificially dried grass are a true measure of its value for the dairy cow in the amounts in which it has been fed—about 8 lb. per head daily—forming some 25 per cent. of the daily intake of dry matter.

It has, however, the marked advantage over mixture of oil cakes and cereals that it raises the carotene in the milk, increasing the yellow colour of the milk. Apart from the immediate practical importance of this effect, which is considered so desirable by the farmer, the bearing of this improvement on the consumer must not be forgotten. It has been shown<sup>(20)</sup> that the carotene content of milk is correlated with its vitamin A content, and carotene itself is a precursor of this vitamin, so that any improvement in the direction of an increase in carotene content, and hence vitamin A potency, increases the nutritive value of the milk.

The proportion of non-protein nitrogen in artificially dried grass is low, and in fact lower than that normally present in fresh grass, so that it has no bearing on the second aspect of this experiment, *i.e.* the value of these nitrogenous compounds in the feeding of the dairy cow.

The molassed silage and A.I.V. fodder can be considered together. In the first place they were both eaten readily in the amounts fed—30 lb.

per head daily. Both were relatively rich in digestible crude protein and supplied a large proportion of the nitrogen intake of the dairy cows. The non-protein nitrogen in the silages appears to have been utilised efficiently, and formed up to 32 per cent. of the total digestible nitrogen intake in the case of the molassed silage rations and somewhat less in the A.I.V. fodder.

Even on the plane of nutrition adopted, which was lower than that usually advocated, the yield of milk, percentage of butter-fat and yield of butter-fat produced on the diet containing the two silages was not significantly less than that produced on the control ration, a normal winter ration of hay, roots and concentrates.

The molassed silage has had no significant effect on the solids-not-fat in the milk of the cows, but there is an indication that the use of A.I.V. fodder has lowered the percentage in the milk, since this foodstuff has resulted in a significant depression in solids-not-fat content of the milk as compared with artificially dried grass.

Brouwer (21), among other workers, has investigated the effect of the ingestion of silage made with added mineral acids on the acid-base balance of the blood and urine of dairy cows, and found that the urine was apt to become more acid and, in excess, the blood also, though this could be overcome by the use of an alkaline mineral supplement. The possibility of the change in the acid-base balance having some effect on the secretion of the milk cannot be overlooked, and is deserving of fuller investigation.

There is an indication of a greater loss in body weight, though not a significant one on the rations containing molassed silage and A.I.V. fodder as compared with the other treatments, showing that the animals had drawn on their body reserves to some extent, and the yield of milk shows a tendency to be slightly lower where silage or A.I.V. fodder are used. The A.I.V. fodder also shows the tendency to produce a lower butter-fat yield than any of the other treatments, but no one of these differences is significant.

Since in making up the ration the non-protein nitrogen was presumed to possess the same efficiency as the digestible "true" protein nitrogen and the highest value allotted in the literature to it is 50 per cent. (16), this tendency is not surprising. It may be assumed that in good molassed silage or A.I.V. fodder the non-protein nitrogen is of high value, and that the digestible crude protein is a better measure of the value of the nitrogen than the more usually accepted digestible "true" protein or even the protein equivalent figure.

This is a point of great importance, since it shows that the disadvantages of the protein breakdown which occurs in the silage process have been given undue emphasis in the past. In this respect it confirms the results put forward by Kirsch and Jantzon (5). These results must still depend upon the quality of the silage made. Where large amounts of volatile bases are formed, they may not be so favourable, though Kellner (22) has shown that ammonium acetate, a compound typical of that in which the volatile bases exist in silage, can replace protein to some extent in the ruminant. In well-made ordinary molassed silage, as has been shown in this experiment, it is, however, possible to prevent the breakdown of the protein proceeding towards the excessive formation of volatile bases. The effect of molassed silage and A.I.V. fodder on the carotene content of milk is also of import.

The experiment has shown that molassed silage will raise the carotene content of the milk to the level usually associated with milk from pasture-fed cows, whilst the A.I.V. fodder, though it fell off in its efficacy in this respect during the last month, will bring about the same result. A previous experiment over a long period (18) showed that A.I.V. fodder was effective in this regard. An allowance of 30 lb. per head daily of good molassed silage or A.I.V. fodder will keep the colour of the milk at a high level throughout the winter.

It was noted earlier in this paper (Table IX) that there were minor variations in the amounts of nutrients actually fed, as compared with the standards aimed at. The effect of such variations on the foregoing results must be considered.

Although none of the results for milk yield, butter-fat content or live weight, for the different treatments, differed from those for the control ration by a significant amount, at the 20 to 1 level of odds, it is noteworthy that the nearest approach to such a significant difference was shown by the A.I.V. fodder treatment (9.2 per cent. lower milk yield), and that it was for that treatment that there was the largest deficit in starch equivalent actually fed, as compared with the control (5.9 per cent.). It is reasonable to suppose that had there not been this deficit in the amount of starch equivalent fed in the A.I.V. fodder treatment, the difference in milk yield and in percentage of butter-fat, with this treatment, would have been smaller, and still more insignificant, and there would have been little or no difference between A.I.V. fodder and molassed silage in these respects.

In the case of the dried grass treatment, in spite of the fact that there was a deficit of 8.0 per cent. of digestible crude protein fed, in comparison

with the control, the values for milk yield, butter-fat content and live weight differed from the control less than did the molasses silage or A.I.V. fodder, and highly insignificantly so (0.4 per cent. lower milk yield). Had this deficit in the supply of digestible crude protein not occurred, it is highly probable that even the small and insignificant difference between the results for the dried grass and the control treatments would have disappeared.

Thus the conclusion that, in respect of their effect on milk yield, butter-fat content and live weight, all of the rations compared were equivalent, is strengthened by the above consideration of the divergences between the standard of feeding aimed at and actually achieved, inasmuch as a correction for such divergences would tend still further to reduce the already insignificant differences between the results for the different experimental rations and for the control ration.

In conclusion, it should be emphasised that in this experiment the foodstuffs under comparison have not supplied much more than half the total nutrients fed. Under these conditions, the value of the non-protein nitrogen was high, and in calculating the ration the value of the nitrogenous compounds can be based on the digestible crude protein. This point has recently been stressed by Kirsch<sup>(24)</sup>, who states that the non-protein nitrogen can be given full value where silage does not form more than half of the dry matter of the ration. Further work is still needed to ascertain whether the same relative values still hold good when a larger proportion or even the whole of the ration consists of silage.

#### SUMMARY

An experiment has been carried out with dairy cows to measure the value of artificially dried grass, silage made with added molasses and A.I.V. fodder (silage made with mineral acid in quantity sufficient to bring the pH of the mass to between 3.0 and 4.0) in the diet, on the yield and quality of the milk. A preliminary period of 3 weeks when all cows were on the control diet was followed by an experimental period of 17 weeks. There were four treatments: a control ration consisting of hay, roots and concentrates; one in which artificially dried grass replaced part of the concentrates; a third in which molassed silage was fed; and a fourth including A.I.V. fodder. There were originally five blocks of four cows each, the four treatments being randomised in each block, each block of cows standing together in the cowshed to eliminate any possible effect of position. One block went dry after 8 weeks, but the remaining four blocks completed the experiment.

The basis of equivalence of the rations was such that each treatment should supply equal amounts of starch equivalent and digestible crude protein for maintenance and the production of milk.

The artificially dried grass, molassed silage and A.I.V. fodder were all of good quality, high digestibility, and high crude protein content (17–20 per cent. of the dry matter), and supplied approximately half of the total digestible crude protein in the ration. The non-protein nitrogen of the molassed silage supplied 32 per cent. of the total digestible crude protein nitrogen intake, whilst in the A.I.V. fodder it supplied 25 per cent. In addition, the mangolds, fed at a constant rate to all cows, provided some 10 per cent. of the digestible crude protein in the form of non-protein nitrogen compounds. Approximately 8 lb. of dried grass and 30 lb. of molassed silage or A.I.V. fodder were fed per head daily.

A statistical analysis of the milk yields, butter-fat yields, butter-fat percentages and changes in live weight of the cows by the method of covariance showed no significant differences between the four treatments. The A.I.V. fodder diet resulted in a significant depression of the percentage of solids-not-fat in the milk as compared with artificially dried grass, but none of the other treatments showed any significant difference. The artificially dried grass treatment was the only one to show an average percentage of solids-not-fat in the milk in excess of the presumptive standard of 8.5 per cent. The artificially dried grass, at the level fed, replaced an equivalent quantity of starch and protein equivalent in concentrates.

The value of the non-protein nitrogen in molassed silage and A.I.V. fodder is high at the levels fed, and the digestible crude protein content of such fodders is a better guide to the feeding value of the nitrogen than the digestible true protein or protein equivalent figures.

The disadvantage of the protein breakdown in silage has been over-emphasised in the past; the products are of high nutritive value for dairy cows, though this does not refer to bad samples of silage in which the breakdown proceeds to the stage of excessive volatile base formation. Artificially dried grass, molassed silage and A.I.V. fodder in the amounts used in this experiment raise the carotene content of milk, and hence its vitamin A potency and yellow colour, to a level similar to that of the average of the grazing season for pasture-fed cows, though for some reason, not capable of explanation in view of previous experiments, the A.I.V. fodder was not so efficient as the other two foodstuffs. Further experiments in which the amounts of artificially dried grass, molassed silage and A.I.V. fodder are increased, so as to provide the major part of the nutrients, are necessary to complete the picture.

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# THE ERADICATION OF WEEDS IN CEREAL CROPS BY SULPHURIC ACID AND OTHER COMPOUNDS

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## INTRODUCTION

EXPERIMENTS on the use of sulphuric acid solutions for the destruction of annual weeds in cereal crops were started at Jealott's Hill in 1932. The results of the earlier work have already been reported by the senior author(2). In the present study, which covers the years 1934 and 1935, the scope of the investigation has been widened, while a larger number of trials has been carried out in each year. In addition to sulphuric acid, solutions of nitric acid, sodium hydrogen sulphate, ammonium hydrogen sulphate and ammonium thiocyanate have been tried.

The earlier experiments showed that sulphuric acid at concentrations stated by Rabaté(6) and Korsmo(5) to give a good control of *Papaver Rhoeas* on the Continent were ineffective in this country. It was observed that the hairs in part prevented the spray coming in direct contact with the foliage. In the present investigation suitable wetting agents have been added to the spray in order to increase its covering power. In addition, spraying at various stages of growth has also been carried out, since it was thought that the resistance to sulphuric acid increased with age.

During 1934 and 1935 the weather conditions were not suitable for the optimum growth of cereal crops. In 1934, from April to August, the rainfall was low over the southern half of England, May being an exceptionally dry month. The lack of available moisture in the soil resulted in a low level of yield, more particularly in the case of those experiments on spring-sown cereals. In 1935 the rainfall was above the average in April and June, but the exceptional hot weather prior to harvest led to a very rapid ripening of the grain. In at least one of the experiments on heavy clay soils the wet spring was detrimental to the establishment of the crop. The level of yield obtained in the 1935 experiments, although not exceptional, was, however, considerably above that of 1934.

## EXPERIMENTAL RESULTS

*Experimental technique.* All the experiments consisted of random blocks; the data are therefore capable of statistical treatment according to Fisher's methods(4). The replication varied from three- to fivefold, while in the more elaborate experiments several sets of controls were included. The plot size ranged from 0.01 to 0.02 acre with the exception of two trials in which smaller plots were used. The solutions were applied by means of a Vermorel Plombé Knapsack sprayer with a "three-nozzle" lance; the rate of application was 100 gallons per acre.

Unless it is otherwise stated in the text, the spraying was carried out when the weed species was in the young seedling stage, in general before the fourth true leaf had developed. In the case of experiments on spring cereals, this stage had generally been reached before the cereal was much over 6 in. high, and before active tillering had started. In the experiments on winter wheat, the crop was sometimes more advanced in growth.

The wetting agents employed to increase the covering power of the spray were proprietary products, namely, Agral 1 and Agral 2. These substances had the great advantage of being comparatively stable even in concentrations of 30.0 per cent. sulphuric acid. In all the 1934 field trials and some of the 1935 experiments Agral 1 was used at a concentration of 0.1 per cent. In the later experiments, the newer and more effective Agral 2 was tested at concentrations varying from 0.1 to 0.025 per cent.

The methods of estimating the degree of control brought about by the various treatments have been described previously(2). In the majority of experiments, observations were carried out some 2-3 weeks after spraying. The density was estimated by counting the number of individuals in some 10-30 quadrats chosen at random on each plot. The difference in density between treated and untreated plots was taken as a measure of the control. In other experiments estimates of density were obtained before and after spraying. In the majority of experiments, in addition to determining the degree of weed control brought about by the various treatments, the yield of the cereal crop was also obtained.

*The control of Brassica arvensis (yellow charlock).* The experiments on the eradication of *B. arvensis* can be divided conveniently into two series, namely the 1934 and 1935 trials. In 1934 a number of substances at varying concentrations was investigated, while the effect of adding a wetting agent (Agral 1) was also studied. In the 1935 experiments sulphuric acid solutions alone were used.

Table I. *Control of Brassica arvensis*. 1934 experiments

Treatment	Centre									
	Jealott's Hill, Berks				Wicken, Beds		Radcliffe-on-Trent, Nottingham		Bury St Edmunds, Suffolk	
	I		II							
H <sub>2</sub> SO <sub>4</sub> *: 6.2%	—	—	82.7	1.18	—	—	—	—	—	—
6.2% + Agral 1 0.1%	—	—	94.1	0.46	—	—	—	—	—	—
9.2%	—	—	95.5	0.55	94.6	0.97	87.8	1.15	91.8	0.81
HNO <sub>3</sub> : 7.9%	—	—	87.4	1.02	—	—	—	—	—	—
7.9% + Agral 1 0.1%	—	—	88.2	0.92	—	—	—	—	—	—
11.7%	—	—	95.7	0.54	97.5	0.78	93.7	0.82	95.5	0.58
NaHSO <sub>4</sub> : 10%	67.4	2.13	—	—	—	—	—	—	—	—
15%	—	—	76.2	1.39	—	—	—	—	—	—
15% + Agral 1 0.1%	—	—	76.0	1.36	—	—	—	—	—	—
22.5%	—	—	80.1	1.24	95.2	1.07	90.5	0.99	—	—
NH <sub>4</sub> HSO <sub>4</sub> : 14.4%	—	—	81.3	1.23	—	—	—	—	—	—
14.4% + Agral 1	—	—	94.0	0.69	—	—	—	—	—	—
0.1%	—	—	—	—	—	—	—	—	—	—
21.6%	—	—	79.8	1.27	91.0	1.44	88.5	1.14	—	—
NH <sub>4</sub> CNS: 3.0%	68.1	2.04	—	—	—	—	—	—	—	—
3.0% + Agral 1	71.3	1.98	—	—	—	—	—	—	—	—
0.1%	—	—	—	—	—	—	—	—	—	—
3.0% + Agral 1	75.4	1.80	—	—	—	—	—	—	—	—
0.1% + NaHSO <sub>4</sub>	—	—	—	—	—	—	—	—	—	—
10%	—	—	—	—	—	—	—	—	—	—
3.0% + NaHSO <sub>4</sub>	73.8	1.88	—	—	—	—	—	—	—	—
10%	—	—	—	—	—	—	—	—	—	—
7.0%	89.4	1.20	—	—	—	—	—	—	—	—
Significant difference ( $P=0.05$ )	—	0.62	—	0.415	—	0.75	—	0.59	—	0.19

\* Throughout this paper the strengths of solutions are given as weight volume percentages, e.g. 6.2 per cent. H<sub>2</sub>SO<sub>4</sub> means a solution of which 100 litres contain 6.2 kg. pure H<sub>2</sub>SO<sub>4</sub>. In agricultural practice it is simpler to make up solutions of sulphuric acid on a volume volume basis of concentration. Rabaté in his pioneer work expressed the concentrations as a percentage by volume of pure sulphuric acid, thus the concentrations on a weight volume basis of 9.2, 13.8 and 18.4 per cent. (*vide* Table I, etc.) correspond to 5, 7.5 and 10 per cent. strengths on a volume volume basis. In England the cheapest form of sulphuric acid is brown oil of vitriol (77 per cent. H<sub>2</sub>SO<sub>4</sub>) and the concentrations corresponding to solutions of 9.2, 13.8, 18.4, 20.7 and 27.6 per cent. are on a volume volume basis for brown oil of vitriol approximately 6.5, 10, 13, 15 and 20 per cent.

In Table I are given the results from the five 1934 experiments. The figures in heavy type refer to the percentage control brought about by the various treatments. Since the variance of the data for the plant counts is discontinuous, it is necessary in the statistical analysis of the data to work with the square roots of the estimates of density (plants per square foot). The mean square roots of density for each treatment are given in lighter type for each centre. The calculated significant differences expressed in terms of the square root of the density do not include the control data, since in every case the reduction in weed population brought about by the various treatments is highly significant ( $P < 0.01$ ).

From the figures in Table I it is seen that a 9.2 per cent. concentration

of sulphuric acid has given, as in previous years (2), a high degree of control. Nitric acid at an equivalent concentration (11.7 per cent.) has brought about equally good results in three trials, and is significantly better in a fourth. Solutions of sodium hydrogen sulphate (22.5 per cent.) and ammonium hydrogen sulphate (21.6 per cent.) were similar in their efficiency to sulphuric acid in two trials, but less effective in a third. Reducing the concentration of the solution by one-third in the case of sulphuric and nitric acids has led to a smaller degree of control, but has had no effect in the case of ammonium and sodium hydrogen sulphates. The addition of Agral 1 at these lower concentrations has increased the efficiency for sulphuric acid and ammonium hydrogen sulphate but not for nitric acid and sodium hydrogen sulphate. Ammonium thiocyanate solutions of 3-7 per cent. concentrations have not given results comparable with sulphuric acid in the other trials. The addition of Agral 1 or 10 per cent. sodium hydrogen sulphate alone or together has not increased the effectiveness of the 3.0 per cent. solution.

During the summer of 1934 the authors inspected a few farms where spraying with 9.2 per cent. sulphuric acid on a large scale had not given such good results as those obtained experimentally. At some farms it was found that in addition to *B. arvensis* (Koch) there was also present *B. arvensis* var. *orientalis*. It was thought that the unsatisfactory control might be due to the greater resistance of this variety to spraying with sulphuric acid. In 1935, therefore, spraying was carried out at two centres where *B. arvensis* var. *orientalis* had been found in the previous year, while seed collected in 1934 from one of the farms was sown at Jealott's Hill. The results of these investigations are given in Table II. The estimates of error have been omitted, since none of the differences between treatments was significant. The differences, however, between the control and any treatment are highly significant ( $P < 0.01$ ).

The data in Table II show that at two centres where *B. arvensis* var. *orientalis* was growing, the standard concentration of 9.2 per cent. has

Table II. *Percentage control of Brassica arvensis. 1935 experiments*

Centre	Treatment Sulphuric acid concentrations (%)			
	9.2	11.5	13.8	18.4
Jealott's Hill (Berks)*	100.0	100.0	100.0	100.0
North Petherton (Somerset)*	98.3	99.3	99.7	—
Ilminster (Somerset)*	78.8	—	—	—
Medmenham (Bucks)	85.0	—	—	—
Jealott's Hill (Berks)	80.0	—	—	—

\* At these centres besides *B. arvensis* (Koch), *B. arvensis* var. *orientalis* was also present.

given as good results as the higher concentrations. In the third experiment, the degree of control obtained with the 9.2 per cent. concentration was somewhat less. In the case of North Petherton and Jealott's Hill, spraying was carried out when the seedlings were young. In the third field trial (Ilminster) at least 4-6 leaves had formed before spraying took place. It was noticed that those plants which survived in this experiment had regenerated shoots from the base of the stem, which at this stage showed considerable thickening. In the second Jealott's Hill experiment, where the plants were again somewhat advanced at the time of spraying, this regeneration also occurred. At Medmenham spraying was carried out in a high wind; in consequence some of the *B. arvensis* seedlings were protected from the spray by the cereal crop.

*Relation between eradication of Brassica arvensis and crop yield.* The yield data for three of the 1934 experiments and four of the 1935 experiments are given in Tables III and IV. In 1934 the eradication of *B. arvensis* by sulphuric acid has led to a significant increase at only one centre (Bury St Edmunds), while at Wicken sulphuric acid has decreased the yield. Treatment with either ammonium or sodium hydrogen sulphate has brought about no change in yield at two centres. Spraying with nitric instead of sulphuric acid has significantly increased the yield in two out of three experiments. At Bury St Edmunds top dressing with nitrochalk after spraying with sulphuric acid has given an equally good result as spraying with nitric acid.

Table III. 1934 experiments. *Brassica arvensis*

Centre	Crop	Variety	Density of <i>B.</i> <i>arvensis</i> on control (plants per sq. ft.)	Yield of grain (cwt. per acre)						Sig. diff. ( <i>P</i> = 0.05)
				Con- trol	9.2% $H_2SO_4$	9.2% + ni- $H_2SO_4$ trogen*	11.7% $HNO_3$	22.5% $NaHSO_4$	21.6% $NH_4HSO_4$	
Radcliffe- on-Trent	Spring barley	Spratt Archer	11.72	10.55	10.04	—	13.84	12.44	10.88	5.75
Bury St Edmunds	Spring barley	Spratt Archer	8.2	19.08	21.48	24.44	25.73	—	—	2.18
Wicken	Winter wheat	White Victor	24.25	18.47	15.51	—	21.15	16.63	17.52	2.33

\* The nitrogen applied as a top dressing was in the form of nitrochalk (15.5 per cent. nitrogen) at the rate of 23.25 lb. nitrogen per acre. The nitric acid contained nitrogen equivalent to 26.3 lb. per acre.

In 1935, the presence of *B. arvensis* in the crop has led to a depression in yield at only one centre (North Petherton). At Ilminster and Medmenham, where the experimental errors are unfortunately large, there is

some indication that the suppression of *B. arvensis* has increased the yield. In the Jealott's Hill experiment, the establishment of the oats was adversely affected by the weather conditions, namely waterlogging of the soil in April, and the formation of a hard crust in May due to a period of hot dry weather.

Table IV. 1935 experiments, *Brassica arvensis*

Centre	Crop	Variety	Density of <i>B. arvensis</i> on control (plants per sq. ft.)	Yield of grain (cwt./acre) Treatment				Sig. diff. ( <i>P</i> =0.05)
				Control	H <sub>2</sub> SO <sub>4</sub> (%)			
					9.2	11.5	13.8	
North Petherton	Spring barley	Webb's New Cross	6.04	20.0	22.0	22.57	21.2	2.35
Ilminster	Spring barley	Spratt Archer	8.89	17.03	20.20	—	—	3.64
Medmenham	Spring barley	Spratt Archer	14.55	8.42	11.83	—	—	5.05
Jealott's Hill	Spring oats	Victory	10.57	8.42	8.93	—	—	3.03

*Control of Raphanus raphanistrum (white charlock).* The data for the 1934 experiments are set out in Table V on the same lines as in Table I. The calculated significant differences shown in the table are between treatments, and not between the control and any treatment, since in every case such differences are significant ( $P < 0.01$ ).

Sulphuric acid at a concentration of 13.8–18.4 per cent. has in five experiments given at least a 93.8 per cent. control. Nitric acid at equivalent concentrations (17.7–23.6 per cent.) was slightly but significantly more effective than sulphuric acid in two out of the five experiments. Reducing the concentration of either sulphuric or nitric acid by a third has lowered the efficiency in two out of three experiments. The addition of Agral 1 at these lower concentrations has not significantly increased the effectiveness of sulphuric and nitric acid in any of the experiments. Ammonium hydrogen sulphate at a concentration equivalent to 11.7 per cent. nitric acid led to a similar reduction; sodium hydrogen sulphate was less effective. Agral 1 added to ammonium or sodium hydrogen sulphate has not increased the efficiency. Sodium thiocyanate at 5 per cent. did not give results equal to nitric acid at the optimum concentration (17.7 per cent.), but the 10 per cent. concentration has given a similar reduction to nitric acid. Sodium hydrogen sulphate and ammonium thiocyanate together were no more efficient than ammonium thiocyanate alone.

Table V. *Control of Raphanus raphanistrum. 1934 experiments*

In each experiment, the figures in heavy type refer to the percentage control brought about by the various treatments, while the figures in lighter type are the square roots of the mean densities of the weed population. For further details see p. 370.

Treatment	Centre					
	Jealott's Hill, Berks	Flintham, Notts	Newent, Glos	Dorchester, Oxon	Holyport, Berks*	Milverton, Somerset
H <sub>2</sub> SO <sub>4</sub> : 9.2%	—	—	—	86.6 1.02	—	—
9.2% + Agral 1 0.1%	—	—	—	91.5 0.86	—	—
13.8%	—	96.9 0.53	93.8 0.99	95.5 0.64	—	95.7 0.95
18.4%	—	—	—	—	98.2 0.61	—
HNO <sub>3</sub> : 11.7%	66.3 0.92	—	93.1 1.06	—	—	—
11.7% + Agral 1	83.0 0.87	—	91.4 1.18	—	—	—
0.1%	—	—	—	—	—	—
17.7%	85.6 0.57	99.3 0.31	97.9 0.57	99.6 0.25	—	97.9 0.69
23.6%	—	—	—	—	99.9 0.22	—
NH <sub>4</sub> HSO <sub>4</sub> : 21.6%	80.0 0.68	—	—	—	—	—
21.6% + Agral 1	79.2 0.72	—	—	—	—	—
0.1%	—	—	—	—	—	—
NaHSO <sub>4</sub> : 15.0%	65.5 0.92	—	—	—	—	—
22.6%	57.2 1.06	—	—	—	—	—
22.6% + Agral 1	72.4 0.81	—	—	—	—	—
0.1%	—	—	—	—	—	—
NH <sub>4</sub> CNS: 5%	62.4 0.98	—	—	—	—	—
10%	76.9 0.77	—	—	—	—	—
5% + NaHSO <sub>4</sub>	63.6 0.96	—	—	—	—	—
15.0%	—	—	—	—	—	—
Significant difference between treatments ( <i>P</i> =0.05)	— 0.31	— 0.24	— 0.36	— 0.41	— 0.25	— 0.43

\* The concentrations of sulphuric and nitric acid were increased at this centre on account of the showery weather at the time of spraying.

In 1935 sulphuric acid alone was used for the experiments on the control of *R. raphanistrum*. It is seen from the data in Table VI that a concentration of 13.8 per cent., as in 1934, has given a high degree of control.

Table VI. *Percentage control of Raphanus raphanistrum. 1935 experiments*

Treatment	Centre			
	Hascombe, Surrey	Dorchester, Oxon	Holyport, Berks	Milverton, Somerset
H <sub>2</sub> SO <sub>4</sub> 13.8%	92.25	95.1	96.7	91.9

*The relationship between the eradication of Raphanus raphanistrum and crop yield.* The effect of the various treatments on the yield of the cereal crop was measured in all the 1934 experiments. From the data set out in Table VII it is seen that the eradication of *R. raphanistrum* by

sulphuric acid has led at three centres (Holyport, Milverton, Newent) to very marked and significant increases in yield, namely increases of 58, 61 and 227 per cent. At the three remaining centres where the density of *R. raphanistrum* was considerably less, spraying with sulphuric acid significantly decreased the yield at Flintham, but had no effect at Dorchester. In the Jealott's Hill experiment, where the weed density was so low that the intensity of competition with the oat crop must have been very small, treatment with nitric acid, sodium hydrogen sulphate and ammonium hydrogen sulphate has not significantly affected the yield. The addition of Agral 1 has only depressed the yield in the case of

Table VII. 1934 experiments. *Raphanus raphanistrum*

	Centre					
	Jealott's Hill, Berks (Spring oats—Victory)	Flintham, Notts (Spring oats)	Newent, Glos (Spring wheat—Red Standard)	Dorchester, Oxon (Spring barley)	Holyport, Berks (Spring barley—Plumage 63)	Milverton, Somerset (Spring barley—Spratt Archer)
Density of <i>R. raphanistrum</i> on control (plants per sq. ft.)	2.64	9.70	16.73	8.97	14.68	23.23
Treatment	Yield of grain (cwt. per acre)					
Control	13.53	14.45	4.29	10.71	8.54	13.00
H <sub>2</sub> SO <sub>4</sub> : 9.2 %	—	—	—	10.19	—	—
9.2 % + Agral 1 0.1 %	—	—	—	10.64	—	—
13.8 %	—	12.17	14.06	10.27	—	20.98
13.8 % + nitrochalk*	—	12.56	—	—	—	23.05
18.4 %	—	—	—	—	13.45	—
18.4 % + nitrochalk*	—	—	—	—	14.45	—
HNO <sub>3</sub> : 11.7 %	12.61	—	12.89	—	—	—
11.7 % + Agral 1 0.1 %	12.32	—	13.29	—	—	—
17.7 %	12.28	12.11	12.22	11.83	—	22.27
23.6 %	—	—	—	—	13.32	—
NH <sub>4</sub> HSO <sub>4</sub> : 21.6 %	14.20	—	—	—	—	—
21.6 % + Agral 1 0.1 %	11.22	—	—	—	—	—
NaHSO <sub>4</sub> : 15.0 %	12.39	—	—	—	—	—
22.5 %	13.06	—	—	—	—	—
22.5 % + Agral 1 0.1 %	13.17	—	—	—	—	—
NH <sub>4</sub> CNS: 5 %	7.56	—	—	—	—	—
5 % + NaHSO <sub>4</sub>	9.01	—	—	—	—	—
15.0 %	—	—	—	—	—	—
10 %	5.23	—	—	—	—	—
Significant difference ( <i>P</i> = 0.05)	2.02	1.91	4.27	3.82	3.67	6.90

\* The nitrochalk was added at a rate equivalent to 23.25 lb. of nitrogen per acre. The amount of nitrogen supplied in the nitric acid solutions was 39.5 and 52.6 lb. per acre for the 17.7 and 23.6 per cent. concentrations.



ammonium hydrogen sulphate. On the other hand, all the ammonium thiocyanate treatments have given marked reductions in yield. In the five experiments where nitric acid was compared with sulphuric acid, the yield differences were not significant. The addition of Agral 1 to either sulphuric or nitric acid has not led to a lower yield.

Three of the 1935 experiments were carried out on the same farms as in 1934, at Milverton and Dorchester in the same field, and at Holyport in an adjoining field. It is seen from the data in Table VIII that the yields of the control plots were of the same order at each centre irrespective of difference in crop or locality. The eradication of *R. raphanistrum* has led in each case to very marked increases in yield (74–154 per cent.). It should, however, be noted that the order of these increases bears no direct relation to the density of *R. raphanistrum* on the control areas.

Table VIII. 1935 experiments. *Raphanus raphanistrum*

	Centre			
	Hascombe (Spring oats— Marvellous)	Dorchester (Spring barley —Spratt Archer)	Milverton (Spring barley —Webb's New Cross)	Holyport (Spring oats— Victory)
Density of <i>R. raphanistrum</i> on control (plants per sq. ft.) ... ..	9.14	20.73	34.80	50.46
	Yield of grain (cwt. per acre)			
Treatment				
Control	12.43	13.0	11.67	13.35
Sprayed with H <sub>2</sub> SO <sub>4</sub> (13.8 %)	20.51	22.57	29.71	22.50
Significant difference ( <i>P</i> = 0.05)	2.50	4.36	3.35	1.91

*Control of Papaver Rhoeas (corn poppy).* The previous results (2), in spite of their variability, demonstrated that sulphuric acid solutions of less than 18.4 per cent. were not effective against *P. Rhoeas*. In the 1934 and 1935 experiments concentrations of 18.4 per cent. or more were used, attention in the main being devoted to studying (i) the effect of adding a wetting agent to the solution, and (ii) the relationship between the stage of growth and resistance to spraying.

In Tables IX and X, where the 1934 and 1935 results are set out, it is seen that the addition of either Agral 1 or Agral 2 has significantly increased the efficiency of the spray in only two out of the six trials. In 1934 at Newent, nitric acid was not significantly superior to sulphuric acid, while at Culford Heath increasing the concentration of sulphuric acid from 18.4 to 27.6 per cent. led to a greater control.

Table IX. *Control of Papaver Rhoeas (rosette stage). 1934 experiments*

In each experiment, the figures in heavy type refer to the percentage control brought about by the various treatments, while the figures in lighter type are the square roots of the mean densities of the weed population. For further details see p. 370.

Treatment	Centre					
	Culford Heath, Suffolk		Newent, Glos.		Chapel Brampton, Northants	
H <sub>2</sub> SO <sub>4</sub> : 18·4 %	<b>76·3</b>	1·83	<b>86·3</b>	3·07	<b>79·5</b>	5·63
18·4 % + Agral 1 0·1 %	<b>76·8</b>	1·82	<b>88·8</b>	2·72	<b>90·5</b>	3·60
27·6 %	<b>90·6</b>	1·16	—	—	—	—
27·6 % + Agral 1 0·1 %	<b>94·1</b>	0·89	—	—	—	—
HNO <sub>3</sub> : 23·6 %	—	—	<b>90·5</b>	2·55	—	—
23·6 % + Agral 1 0·1 %	—	—	<b>92·0</b>	2·34	—	—
Significant difference ( $P=0·05$ )	—	0·37	—	0·65	—	1·14

Table X. *Control of Papaver Rhoeas. 1935 experiments*

For each experiment, the figures in heavy type refer to the percentage control brought about by the various treatments, while the figures in lighter type are the square roots of the mean densities of the weed population. For a full explanation see p. 370.

		Centre					
		Jealott's Hill, Berks		Hascombe, Surrey		Wokingham, Berks	
Treatment	Stage of growth						
H <sub>2</sub> SO <sub>4</sub> 18·4 %	Young seedling	<b>91·2</b>	2·27	<b>87·7</b>	3·51	—	—
	Advanced rosette	—	—	—	—	<b>67·5</b>	3·76
	Flower heads formed	<b>22·2</b>	7·74*	—	—	—	—
H <sub>2</sub> SO <sub>4</sub> 18·4 % + Agral 2 0·1 %	Young seedling	<b>95·5</b>	1·37	<b>96·9</b>	1·78	—	—
	Advanced rosette	—	—	—	—	<b>73·4</b>	3·43
Significant difference ( $P=0·05$ )		—	3·04	—	0·84	—	0·82

\* This figure is not significantly different from the control.

In the 1934 experiments, the spray was applied when the *P. Rhoeas* plants showed no well-defined development of the flowering shoot (rosette stage). In 1935, when spraying was carried out at younger and older stages (*vide* Table X), the most effective control was obtained at a time when the first true leaves had just formed (seedling stage), the resistance increasing very markedly with age.

*The relationship between eradication of Papaver Rhoeas and crop yield.* The changes in the yield of the cereal crop following upon the suppression of *P. Rhoeas* are seen in Table XI for the 1934 experiments and two of the 1935 field trials. In only one out of the five experiments has spraying with an 18·4 per cent. concentration of sulphuric acid resulted in a significantly greater yield. The incorporation of a wetting agent in the

solution, in spite of the better control obtained in some cases (*vide* Table IX), has not led to a further increase in yield, but, on the contrary, at Culford Heath, to a decrease. A top dressing of nitrochalk after spraying with acid alone has significantly increased the yield at Hascombe, but not at Chapel Brampton and Wokingham. Similarly a dressing of nitrochalk after spraying with sulphuric acid and Agral has given a higher yield only at Chapel Brampton. At Newent, where sulphuric acid was compared with nitric acid, spraying with the latter resulted in a significantly higher yield.

Table XI. 1934-35 *experiments*. *Papaver Rhoeas*

	Centre				
	Culford Heath (Barley— Spratt Archer)	Newent (Spring wheat— Red Standard)	Chapel Brampton (Spring Oats— Marvellous)	Hascombe (Spring oats— Black Spring Tartar)	Wokingham (Spring wheat)
Density of <i>P. Rhoeas</i> on control (plants per sq. ft.)	14.5	75.8	161.6	108.7	47.7
	Yield of grain (cwt. per acre)				
Treatment					
Control	6.64	5.08	10.30	14.20	5.38
H <sub>2</sub> SO <sub>4</sub> : 18.4 %	7.48	5.13	11.01	14.26	6.29
18.4 % + Agral	6.53	6.75	8.57	14.22	6.45
18.4 % + Agral + nitrogen*	—	—	13.90	17.12	6.67
18.4 % + nitrogen	—	—	11.21	21.52	6.67
27.6 %	6.47	—	—	—	—
27.6 % + Agral	5.97	—	—	—	—
HNO <sub>3</sub> : 23.6 %	—	9.65	—	—	—
23.6 % + Agral	—	8.87	—	—	—
Significant difference ( <i>P</i> = 0.05)	0.54	2.04	3.70	5.05	2.51

\* The nitrogen was applied as nitrochalk at the rate of 23.25 lb. nitrogen per acre. At Newent spraying with nitric acid was equivalent to adding 52.6 lb. nitrogen per acre.

*Control of Chrysanthemum segetum (corn marigold).* Since the leaves of *C. segetum* are covered with a superficial layer of wax, it was thought that a greater control would be obtained if a wetting agent were added to the sulphuric acid. From the data in Table XII it is evident that the addition of Agral has very greatly increased the efficiency of both sulphuric and nitric acid. Solutions of 13.8-18.4 per cent. sulphuric acid to which either Agral 1 or 2 was added have given over a 97 per cent. control in the two Jealott's Hill experiments. At Broadclyst the degree of control was somewhat less, but this in the case of the 13.8 per cent.

concentration was probably due to weather conditions, since rain fell within an hour of spraying.

Table XII. *Control of Chrysanthemum segetum*

In each experiment, the figures in heavy type refer to the percentage control brought about by the various treatments, while the figures in lighter type are the square roots of the mean densities of the weed population. For a full explanation see p. 370.

Treatment	Jealott's Hill				Broadclyst, Devon, 1935	
	1934		1935			
H <sub>2</sub> SO <sub>4</sub> : 9.2% + Agral* 0.1%	—	—	—	—	<b>62.9</b>	4.31
13.8%	<b>72.0</b>	2.63	—	—	—	—
13.8% + Agral 0.1%	<b>97.0</b>	0.79	—	—	<b>84.0</b>	2.78
18.4%	<b>67.4</b>	2.83	<b>63.4</b>	4.29	—	—
18.4% + Agral 0.1%	<b>98.8</b>	0.50	<b>98.2</b>	0.85	—	—
18.4% + Agral 0.05%	—	—	<b>98.6</b>	0.83	—	—
18.4% + Agral 0.025%	—	—	<b>94.1</b>	1.60	—	—
HNO <sub>3</sub> : 17.8%	<b>85.8</b>	1.87	—	—	—	—
17.8% + Agral 0.1%	<b>97.2</b>	0.83	—	—	—	—
Significant difference between treatments ( $P=0.05$ )	—	0.52	—	0.73	—	1.02
Density of <i>C. segetum</i> on control (plants per sq. ft.)						
Yield of grain (cwt. per acre)						
Centre	Control	9.2% H <sub>2</sub> SO <sub>4</sub> + Agral	13.8% H <sub>2</sub> SO <sub>4</sub> + Agral	Sig. diff. ( $P=0.05$ )		
Broadclyst (Barley—Spratt Archer)	14.8	15.8	17.8	2.20		

\* Agral 1 was used in 1934, Agral 2 in 1935.

*Relation between eradication of Chrysanthemum segetum and crop yield.* Data on the influence of *C. segetum* in depressing the yield of the cereal crop are available only for Broadclyst. At Jealott's Hill it was necessary in order to carry out experiments to sow seed of *C. segetum*. The quantity available was insufficient to sow an area large enough for trials in which the yield data could be recorded with accuracy. The data for Broadclyst show (*vide* Table XII) that the more effective control obtained with the 13.8 per cent. concentration has increased the yield by some 20 per cent.

*Control of Anthemis cotula (stinking mayweed).* In the two experiments on *A. cotula*, the stage of growth was somewhat different at the two centres. At Kettering the plants at the time of spraying (May) were several inches high, and only a partial control was obtained (*vide* Table XIII). At Ham, the plants were much smaller when spraying was carried out in March, and a good control was obtained with a high concentration of sulphuric acid (27.6 per cent.), particularly when Agral was added.

Table XIII. *Control of Anthemis cotula*

For each experiment, the figures in heavy type refer to the percentage control brought about by the various treatments, while the figures in lighter type are the square roots of the mean densities of the weed population. For further details see p. 370.

Density on control (plants per sq. ft.)	Treatment	Percentage control				Yield of grain (cwt. per acre)	
		Kettering, Northants		Ham, Wilts		Kettering (Winter oats—Garton's Unique)	Ham (Winter wheat—Yeoman)
		—	—	—	—	6.1	15.4
Control		—	—	—	—	11.55	11.70
H <sub>2</sub> SO <sub>4</sub> : 13.4 %		<b>57.4</b>	1.56	<b>26.0</b>	3.35	11.27	14.35
13.4 % + Agral 1		<b>51.4</b>	1.70	—	—	9.88	—
0.1 %		—	—	—	—	—	—
18.4 %		<b>55.3</b>	1.63	—	—	9.32	—
18.4 % + Agral 1		<b>47.7</b>	1.71	—	—	8.54	—
0.1 %		—	—	—	—	—	—
20.7 %		—	—	<b>51.7</b>	2.69	—	15.33
27.6 %		—	—	<b>73.2</b>	1.97	—	16.47
27.6 % + Agral 1		—	—	<b>87.6</b>	1.37	—	17.10
0.1 %		—	—	—	—	—	—
Significant difference ( <i>P</i> = 0.05)		—	0.59	—	0.58	2.83	3.11

*The relation between eradication of Anthemis cotula and crop yield.*

At Kettering, the partial control of *A. cotula* has not led to any increase in yield (Table XIII), but rather to a depression after spraying with an 18.4 per cent. concentration of sulphuric acid and Agral. This depression may in part have been due to the depredations of rabbits preventing the oats from recovering after spraying. At Ham, all treatments have significantly increased the yield of the wheat by some 22–46 per cent.

*Control of Scandix Pecten-Veneris (shepherd's needle).* In the single experiment on the eradication of *S. Pecten-Veneris*, the plants at the time of spraying (March) were several inches high, having germinated in the previous autumn of 1934. From the data in Table XIV it is seen that sulphuric acid even at a concentration of 27.6 per cent. and with the addition of Agral has led only to a partial control (60 per cent.). Nevertheless, all the spraying treatments have resulted in significant increases in yield, varying from 46 to 86 per cent. The addition of Agral 1 (0.1 per cent.) to sulphuric acid, even at a concentration of 27.6 per cent., has not depressed the yield.

*Susceptibility of barley to spraying with sulphuric acid.* In the previous investigation (2) it was found that the yield of barley sown on "clean"

Table XIV. *Control of Scandix Pecten-Veneris*

Centre	Treatment					
	Control	13.8% H <sub>2</sub> SO <sub>4</sub>	20.7% H <sub>2</sub> SO <sub>4</sub>	27.6% H <sub>2</sub> SO <sub>4</sub>	27.6% H <sub>2</sub> SO <sub>4</sub> + Agral 1 0.1%	Sig. diff. ( <i>P</i> = 0.05)
Badminton, Glos (Winter wheat—Square Head's Master)	—	24.6	42.2	61.5	60.5	—
Percentage control	—	24.6	42.2	61.5	60.5	—
Square root of density (plants per sq. ft.)	—*	3.34	3.14	2.56	2.59	0.335
Yield of grain (cwt. per acre)	8.19	11.81	13.44	14.98	15.25	2.83

\* The density of *S. Pecten-Veneris* on the control plots was 17.2 plants per sq. ft.

Table XV. *Influence of spraying on yield of barley (Spratt Archer)*

		Jealott's Hill Yield of grain (cwt. per acre)	
Treatment		1934	1935
Control		23.73	18.92
H <sub>2</sub> SO <sub>4</sub> : 13.8%		19.23	16.63
18.4%		16.67	—
13.8% + nitrochalk*		18.13	—
18.4% + nitrochalk		20.18	—
13.8% + Agral 0.1%†		17.91	13.74
18.4% + Agral 0.1%		15.41	—
13.8% + Agral 0.1% + nitrochalk		17.62	20.93
18.4% + Agral 0.1% + nitrochalk		17.14	—
HNO <sub>3</sub> : 17.7%		21.90	29.32
23.6%		19.46	—
17.7% + Agral 0.1%		19.84	—
23.6% + Agral 0.1%		18.50	—
Significant difference between			
(i) Treatment and control		2.08	—
(ii) Treatments		2.73	2.74
		Z	Value of Z ( <i>P</i> = 0.05)
Significant acid effect (1934 experiment)		0.7332	0.7045
17.7% HNO <sub>3</sub> v. 23.6% HNO <sub>3</sub>			
Significant Agral effect (1934 experiment)		0.8114	0.7045
H <sub>2</sub> SO <sub>4</sub> v. H <sub>2</sub> SO <sub>4</sub> + Agral			
Significant interaction (1934 experiment)		0.8951	0.7045
H <sub>2</sub> SO <sub>4</sub> × nitrochalk			

\* The nitrochalk was applied at the rate of 23.25 lb. nitrogen per acre. The amount of nitrogen applied in the nitric acid was equivalent to 39.5 and 52.6 lb. per acre for the 17.7 and 23.6 per cent. concentrations.

† Agral 1 was used in 1934, Agral 2 in 1935.

land was not depressed by spraying with sulphuric acid solutions unless the crop was more than 6 in. high. In continuation of this work, further experiments were carried out in 1934 and 1935. In 1934 the crop was almost entirely free from weeds, but in 1935 a few plants of *B. arvensis* were present (density less than one plant per square foot). In both years the spray was applied when the barley was some 6 in. high. The results of the two experiments are given in Table XV.

The yield figures in Table XV show that in 1934, with the exception of 17.7 per cent. nitric acid, all the treatments have depressed the yield to a varying degree. Sulphuric acid has lowered the yield more than nitric acid. The greater depression due to spraying with the higher concentrations of nitric acid is significant (*vide* effect, Table XV). The corresponding effect with sulphuric acid when averaged over plots with and without nitrochalk is not significant. There is, however, a significant interaction of strength of sulphuric acid with nitrochalk, the effect of the strength of sulphuric acid being, like nitric acid, significant when the plots without nitrochalk are considered separately. The addition of Agral to the sulphuric acid has significantly depressed the yield, and there is evidence of the same effect with nitric acid, though in the latter case it is not significant.

In 1935 nitric acid, in contrast to the results of 1934, has given a higher yield than the control, while sulphuric acid has not depressed the yield. The addition of Agral to the sulphuric acid has again brought about a marked reduction, but top dressing with nitrochalk has compensated for the depression due to the addition of Agral.

*The influence of sulphuric acid spraying on the malting quality of barley.* It was found in the earlier investigation that the malting quality of barley was not affected by spraying the crop with 9.2–18.4 per cent. concentrations of sulphuric acid. In the present experiments the effect of adding a wetting agent or the substitution of nitric acid for sulphuric acid have also been studied.

The weather conditions during 1934 and 1935 were not suitable for the production of high-quality malting samples, since during the ripening period in both years the weather was exceptionally hot and dry.

Although on the basis of Bishop and Day's researches<sup>(3)</sup> the nitrogen content and grain weight data (*vide* Table XVI) indicate that many of the samples were of high malting quality, the absence of "colour" and plumpness precluded a high valuation in the market.

It is seen in Table XVI that spraying with sulphuric acid has not affected the value of the sample in any of the four 1935 trials. Spraying

Table XVI. *Influence of spraying on malting quality of barley*

Treatment	Nitrogen content of grain %	1000 grain weight gm.	Market value* shillings per quarter
<b>Jealott's Hill, 1934:</b>			
Control	1.57	37.3	37
H <sub>2</sub> SO <sub>4</sub> : 13.8 %	1.60	36.3	37
18.4 %	1.72	37.1	36
13.8 % + Agral	1.68	36.9	36
18.4 % + Agral	1.64	37.2	36
13.8 % + Agral + nitrochalk†	2.04	40.8	32
18.4 % + Agral + nitrochalk	1.98	40.2	32
13.8 % + nitrochalk	2.05	40.0	32
18.4 % + nitrochalk	2.00	40.0	32
HNO <sub>3</sub> : 17.7 %	2.07	39.6	32
23.6 %	2.29	39.7	31
17.7 % + Agral	2.10	40.0	32
23.6 % + Agral	2.43	40.1	30
<b>Jealott's Hill, 1935:</b>			
Control	1.34	38.3	30
H <sub>2</sub> SO <sub>4</sub> : 13.8 %	1.40	36.8	30
13.8 % + Agral	1.41	36.3	30
13.8 % + Agral + nitrochalk	1.47	37.0	27
HNO <sub>3</sub> : 17.7 %	1.41	36.6	27
<b>Broadclyst, 1935:</b>			
Control	1.29	30.6	34
H <sub>2</sub> SO <sub>4</sub> : 9.2 % + Agral	1.34	31.1	34
13.8 % + Agral	1.43	33.7	34
9.2 % + Agral + nitrochalk	1.55	32.0	32
13.8 % + Agral + nitrochalk	1.64	33.1	30
<b>Dorchester, 1934:</b>			
Control	1.67	36.6	37
H <sub>2</sub> SO <sub>4</sub> : 9.2 %	1.81	39.3	34
9.2 % + Agral	1.95	38.3	34
13.8 %	1.82	39.9	34
HNO <sub>3</sub> : 17.7 %	2.40	36.6	32
<b>Dorchester, 1935:</b>			
Control	1.37	32.4	35
H <sub>2</sub> SO <sub>4</sub> : 13.8 %	1.32	32.7	35
<b>Milverton, 1934:</b>			
Control	1.35	31.4	39
H <sub>2</sub> SO <sub>4</sub> : 13.8 %	1.51	34.3	38
13.8 % + nitrochalk	1.67	33.6	37
HNO <sub>3</sub> : 17.7 %	1.89	33.1	35
<b>Milverton, 1935:</b>			
Control	1.48	31.9	33
H <sub>2</sub> SO <sub>4</sub> : 13.8 %	1.41	37.6	33
<b>Bury St Edmunds, 1934:</b>			
Control	1.71	40.1	37
H <sub>2</sub> SO <sub>4</sub> : 9.2 %	1.83	38.9	35
9.2 % + nitrochalk	1.98	40.2	33
HNO <sub>3</sub> : 11.7 %	2.03	41.9	33
<b>Ilminster, 1935:</b>			
Control	1.37	32.4	35
H <sub>2</sub> SO <sub>4</sub> : 9.2 %	1.32	32.7	35

\* Market valuations based on prices ruling January 17 and December 31, 1935 in the London Market.

† In all the experiments nitrochalk was applied at a rate equivalent to 23.25 lb. nitrogen per acre.



has, however, decreased the value by a small extent in two out of the three 1934 experiments. The addition of Agram to the sulphuric acid has not affected the malting quality. Spraying with nitric acid has increased the nitrogen content of the grain, and has led to an appreciable drop in the value of the sample. Top dressing with nitrochalk after spraying with sulphuric acid has also led to a decrease in value.

### DISCUSSION

The results of the present investigation are in agreement with previous findings. Concentrations of sulphuric acid, which were found very effective in the earlier work, have again given a high degree of control. In the nine experiments on *Brassica arvensis*, the average control was 90 per cent. when a 9.2 per cent. concentration was used. With *Raphanus raphanistrum*, a 95 per cent. reduction in density (average of nine experiments) was obtained with a 13.8 per cent. solution. Reducing these concentrations by a third led on the whole to less effective results, and this loss of efficiency was not generally corrected by the addition of a suitable wetting agent to the spray.

Equivalent concentrations of nitric acid have been found slightly more effective than sulphuric acid in controlling *Brassica arvensis* or *Raphanus raphanistrum*, the average figures for the nine experiments being 97.5 per cent. control as against 94.5 per cent. For the control of *Chrysanthemum segetum* this superiority of nitric acid was more marked, but there was no significant difference between the two acids in the single trial on *Papaver Rhoeas* (Newent).

For the eradication of *Brassica arvensis* ammonium hydrogen sulphate and sodium hydrogen sulphate have given results very nearly equal to sulphuric acid. In the case of *Raphanus raphanistrum* equivalent hydrogen concentrations of sodium hydrogen sulphate corresponding to 13.8 per cent. sulphuric acid could not be made up on account of the low solubility of the salt. This difficulty did not occur with ammonium hydrogen sulphate, but by increasing the concentration beyond 21.6 per cent. there was a danger of adding an excessive quantity of nitrogen to the crop. At the concentrations used for the control of *Brassica arvensis* both ammonium and sodium hydrogen sulphate gave inferior results to sulphuric acid at 13.8 per cent. Ammonium thiocyanate at the concentrations investigated compared unfavourably with sulphuric acid for the control of both *Brassica arvensis* and *Raphanus raphanistrum*.

The experiments have shown that for the effective control of *Papaver Rhoeas* spraying must be carried out in the earliest stages of development.

The greater resistance of the plants with increasing age is in direct conflict with Rabaté's contention (6). The addition of the wetting agents (Agral 1 and 2) has given variable results; it is to be noted, however, that where increases in efficiency occurred, the density of *Papaver Rhoeas* was very high (Chapel Brampton, Hascombe). Consistent results in which the incorporation of Agral has led to a greater control have been obtained only in the experiments on *Chrysanthemum segetum*.

On the basis of these findings, it is possible to put forward some conclusions as to the factors involved in the successful control of annual weeds by sulphuric acid. For such species as *Brassica arvensis*, *Raphanus raphanistrum* and *Papaver Rhoeas*, effective control is dependent primarily on the quantity of acid remaining in contact with the tissues, the amount necessary to kill the cells varying with the species. Since in these species the epidermis is neither thickened nor waxy, the addition of a wetting agent is of little benefit except in cases where the density may be so high that the leaves from one plant overlap the other. Here the incorporation of Agral may lead to a greater control owing to the more complete cover of the spray, e.g. *Papaver Rhoeas* at Hascombe and Chapel Brampton. With *Chrysanthemum segetum* the use of a wetting agent leads not only to a greater quantity of solution adhering to the tissues, but also to a quicker penetration through the waxy epidermis. In this connection, a recent paper by Engledow and Woodman (7) is of interest, since they found on lawns that for the effective control of *Polygonum aviculare* and *Trifolium striatum* in the seedling stage it is necessary to incorporate a wetting agent in the ammonium sulphate solution, since the cotyledons have a waxy epidermis.

The elimination of weed competition has led to very variable effects on the yield of the cereal crop. The experiments have shown that the suppression of *Raphanus raphanistrum* may result in increases in yield varying from 0 to 227 per cent. The order of the increase bears no direct relationship to the density of the weed population. Experiments in which the weed density was of the same order have given very different results, e.g. no increase at Flintham and a 65 per cent. higher yield at Hascombe. For the ten experiments, the average increase in yield was 71 per cent., when the average density of *R. raphanistrum* on the control was 19.1 plants per square foot.

The suppression of *Brassica arvensis* has resulted in a significantly higher yield in only two out of the seven experiments, although in two other experiments, where the experimental errors were large, there was some indication of increases in yield. It must, however, be remembered that at none of these centres was the weed density high, the average

being 12.0 plants per sq. ft. as against 19.1 plants in the trials on *Raphanus raphanistrum*. In the case of the *Papaver Rhoeas* this explanation of low weed density cannot account for the small increases in yield obtained as a result of spraying, since in spite of the average density being 81 plants per square foot, a significant increase was obtained at only one out of the five centres. As far as can be judged from the small number of trials on *Anthemis cotula*, *Scandix Pecten-Veneris* and *Chrysanthemum segetum*, the eradication of these species leads to considerably higher yields of grain.

The evidence from this investigation is in agreement with Korsmo's findings (5), namely that nitric acid is not very greatly superior to sulphuric acid in increasing the crop yield. In only two experiments has the difference in yield been at all striking (Jealott's Hill, Newent), although in two other trials (Wicken, Bury) spraying with nitric acid has resulted in slightly greater yields than sulphuric acid. In the remaining seven experiments, however, no differences were obtained. On the other hand, all except one of the experiments were carried out in 1934, which was an exceptionally dry year and one in which water rather than nitrogen supply was the probable factor limiting yield. It would not, therefore, be expected that the differences between sulphuric and nitric acid would be large. Even, however, in normal seasons with the higher concentrations of nitric acid, there is the danger that an excessive quantity of nitrogen may be added to the crop, leading not only to lodging but also in the case of barley to a lowering of the malting quality (*vide* Table XVI). The difficulty of adding excessive nitrogen does not arise in the case of sulphuric acid. If a nitrogenous fertiliser is required the quantity applied can be adjusted according to the crop and the previous manuring.

Spraying with sulphuric or nitric acid has in a few experiments resulted in damage to the crop. At Wicken a 9.2 per cent. concentration of sulphuric acid depressed the yield of winter wheat, yet at Ham and Badminton there is little evidence that a 27.6 per cent. concentration was harmful. At Flintham sulphuric acid (13.8 per cent.) or nitric acid (17.7 per cent.) lowered the yield of spring oats, but the same strength of nitric acid at Jealott's Hill had no adverse effect. Solutions of 5-10 per cent. ammonium thiocyanate, however, in the same trial reduced the yield by 33-66 per cent. Contrasting results were also obtained for sulphuric and nitric acid in the "clean" barley experiments (*vide* Table XV) in the two years 1934 and 1935.

In every experiment where damage was recorded spraying was carried out in 1934 at a time when the weather was exceptionally dry.

It would be expected that this damage might be associated with drought, since the temporary check which occurs after spraying in a wet year may have a permanent effect in a dry year. With the exception of one experiment (Jealott's Hill, 1934, *vide* Table XV), the order of the depression brought about by spraying with sulphuric acid or nitric acid is not large.

Since Aslander<sup>(1)</sup> has already pointed out that the resistance of cereals to damage by sulphuric acid is in part due to the presence of a waxy cuticle, it was realised that the addition of a wetting agent to the spray might result in a depression in yield. There are some twelve experiments in which the effect on the yield of adding Agral can be observed. In three of the experiments (Chapel Brampton, Ham, Hascombe) the position is somewhat complicated, since the addition of Agral increased the efficiency of the spray in controlling *Papaver Rhoeas* or *Anthemis cotula*. It is considered that the order of these increases is not large enough to affect the yield to any great extent. In seven of the remaining trials there was no difference in the percentage control, while in two of the Jealott's Hill experiments on barley, since no weeds were present a direct contrast can be made.

The addition of Agral to 13.8–18.4 per cent. sulphuric acid has led to a significant depression in two out of the four experiments on barley. In the three spring oat trials there was possibly some damage caused by including Agral (Hascombe) with 18.4 per cent. sulphuric acid. Spring wheat was unaffected in three trials where Agral was added to sulphuric acid at a concentration of 18.4 per cent. or to nitric acid at concentrations of 17.7 and 23.6 per cent. In the case of winter wheat, even the addition of Agral to a 27.6 per cent. solution does not appear to have any deleterious effect.

In the previous paper it has already been pointed out that weather conditions in the late spring would appear to play an important part in regard to weed competition. Dry conditions are unfavourable to the growth of the majority of weed species studied, and under these circumstances the suppression of the weeds may not lead to large increases in yield. The data for *Raphanus raphanistrum* tend to confirm this, since the order of the increase in yield obtained as a result of weed suppression in 1935 is greater than in 1934, a dry year. The presence of *R. raphanistrum* in the cereal crop led to the largest depressions in yield. Although the data for *Brassica arvensis* are not strictly comparable with the figures for *Raphanus raphanistrum*, it seems evident that this species cannot compete so successfully with the cereal. *Brassica arvensis*, however, brings about greater decrease in yield than *Papaver Rhoeas*.

It has already been pointed out that the order of the decrease in yield of the cereal is not directly correlated with the density of the weed. The nature of the factors involved in the competition between the weed and the cereal will be discussed in detail in another paper. Briefly these further investigations have shown that competition is primarily for light and nitrogen. The relative importance of these two factors is dependent upon the density of the weed, the weed species and the weather conditions in May and early June.

Finally it is concluded that in agricultural practice sulphuric acid is the most suitable compound for the destruction of annual weeds. Although nitric acid may be both slightly more effective in controlling the various weed species and increasing the yield, these advantages are more than offset by its greater cost, corrosive properties on metals and its liability both to cause lodging and to lower the malting quality of barley. The inclusion of wetting agents such as Agral 1 or 2 is only warranted for the destruction of such weeds as *Chrysanthemum segetum*, while this treatment, particularly in the cases where the crop is barley, should be followed by a nitrogenous top dressing in a normal year.

#### SUMMARY

In continuation of earlier work (2) on the destruction of annual weeds in cereal crops some thirty-four replicated experiments have been carried out during 1934 and 1935 in widely different localities. Besides sulphuric acid, nitric acid, ammonium and sodium hydrogen sulphates and ammonium thiocyanate were tested, while the effect of a wetting agent was also studied.

In every experiment the solution was applied by means of a knapsack sprayer at the rate of 100 gallons per acre. In general at the time of spraying the weeds were in the seedling stage, and the cereal crop not more than 6 in. high. Counts of the weed density were made in order to estimate the degree of control, while in the majority of the trials the yield of the cereal was determined.

Sulphuric acid at a concentration of 9.2 per cent. (1 gm.  $H_2SO_4$  per 100 c.c.) gave a 90 per cent. control of *Brassica arvensis* (average of nine experiments) and when a 13.8 per cent. strength was used it gave a 95 per cent. control of *Raphanus raphanistrum* (average of eight experiments). Nitric acid at equivalent concentrations was slightly but significantly more effective in three out of nine trials for the eradication of these two species. Ammonium hydrogen sulphate (21.6 per cent.),

sodium hydrogen sulphate (23·6 per cent.) or ammonium thiocyanate (3–10 per cent.) did not, on the whole, compare favourably with sulphuric acid. Decreasing the concentrations of sulphuric acid by a third resulted in a less effective destruction of *Brassica arvensis* or *Raphanus raphanistrum*. This loss of efficiency at the lower concentrations was not corrected by the addition of a wetting agent (Agral 1 at 0·1 per cent.); similar results were obtained for nitric acid.

The resistance of *Papaver Rhoeas* to sulphuric acid increases with age. In the youngest stage of growth over a 90 per cent. control was obtained with a concentration of 18·4 per cent. In two out of six experiments, the addition of a wetting agent gave a higher "kill". An effective control of *Chrysanthemum segetum* was obtained with a 13·8–18·4 per cent. concentration of sulphuric acid *only* when the wetting agent Agral was included in the spray. A 27·6 per cent. strength partially eradicated both *Scandix Pecten-Veneris* and *Anthemis cotula* (61·5 and 73·2 per cent. control). The addition of Agral led to a greater suppression (87·6 per cent.) of *A. cotula*.

The suppression of *Brassica arvensis* by sulphuric acid increased the yield of the cereal by 12–13 per cent. in two out of the seven trials. The eradication of *Raphanus raphanistrum* has resulted in a higher yield in seven out of the ten experiments, the increase ranging from 58 to 227 per cent. In only one out of the five experiments on *Papaver Rhoeas* did spraying with sulphuric acid lead to a higher yield. The control of *Chrysanthemum segetum*, *Anthemis cotula* and the partial control of *Scandix Pecten-Veneris* gave increases of 20, 46 and 86 per cent. respectively in the three experiments.

Nitric acid, in comparison with sulphuric acid, gave greater yields in five out of ten trials. In four out of these five trials the addition of nitrochalk (22·5 lb. nitrogen per acre) after spraying with sulphuric acid increased the yield to the same extent as nitric acid.

Sulphuric acid does not normally depress the crop yield. In a very dry spring some damage may occasionally occur, depressions of 15·8, 16·0 and 29·7 per cent. were recorded. The addition of Agral to sulphuric acid depressed the yield by 6·9–17·0 per cent. in three out of twelve experiments; barley would appear to be more susceptible than oats or wheat. Ammonium thiocyanate lowered the yield of oats by 33·4–61·4 per cent. The malting quality of barley was not appreciably lowered by spraying with sulphuric acid or sulphuric acid and Agral, but nitric acid diminished the value of the sample.

It is concluded that the order of the increase in yield of the cereal due

to the suppression of weeds is dependent upon the weed species, but is not directly correlated with the density. In a dry spring competition is less severe than in a wet spring.

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# THE EFFECT OF APPLYING A NITROGENOUS FERTILISER TO WHEAT AT DIFFERENT STAGES OF GROWTH

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(With Five Text-figures)

## INTRODUCTION

THE relation between the time of application of nitrogenous fertilisers to wheat and the yield response produced has been the subject of a large number of field experiments in many countries. The results of different investigators show considerable disagreement, and this fact, together with the variations in response found in different growing seasons, draws attention to the very obvious importance of meteorological factors in the consideration of this problem. Of these factors, the amount and distribution of rainfall during the growing season is undoubtedly of primary importance. Rainfall may affect the response of field crops to nitrogenous fertilisers in two ways; excessive rainfall may leach the nitrogen applied away from those regions of the soil from which it can be absorbed by the roots of the crop, while if the rainfall is subnormal the water supply available to the crop may become a limiting factor to growth, so that additional nitrogen in the soil cannot be utilised, even if it is taken up by the crop. The differential incidence of these opposite effects at different periods in the growing season is itself capable of inducing variations in the response to nitrogenous fertilisers applied at varying stages of growth. Thus, the problem can be considered from two aspects: (*a*) the ability of the wheat plant to take up and utilise in growth a given quantity of nitrogenous fertiliser presented at different times to, and remaining within the sphere of activity of, its roots; (*b*) the effect of meteorological factors, particularly of rainfall, in altering the availability to the plant of the nitrogen applied.

In field experiments these two aspects cannot be separated. But it is easy to eliminate the factor of excessive or deficient water supply in pot culture by ensuring that while an adequate water supply is given at all times, it is never so great as to cause drainage from the pots.



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The object of the present work, therefore, was to investigate the ability of the wheat plant to utilise nitrogen given at different stages of growth, with the major disturbing field factor of variable water supply eliminated.

### DETAILS OF THE EXPERIMENT

A pure line of Yeoman II wheat was used. The plants were grown in unmanured Rothamsted soil from Broadbalk field, mixed with 10 per cent. of coarse sand to prevent compacting of the soil by watering. 28 lb. of the soil-sand mixture was weighed out into each pot, and a basal dressing consisting of 0.65 gm. of  $\text{KH}_2\text{PO}_4$  and 0.2 gm. of  $\text{K}_2\text{SO}_4$  was applied, in the form of an aqueous solution which was pipetted on to the dry soil and well mixed with it. The seed was soaked in 0.2 per cent. formalin solution for 4 hours and then allowed to dry before sowing. Nine seeds selected to weigh between 0.05 and 0.06 gm. were planted in each pot at a depth of 1 in. The seed treatment had apparently no adverse effect on germination, since 97 per cent. of the seeds germinated. The number of plants per pot was reduced to six soon after germination.

### *Treatments*

Sodium nitrate was applied at three rates:

#### Treatment

1 N	1.03 gm. $\text{NaNO}_3$ per pot = 0.17 gm. N
2 N	2.06 gm. $\text{NaNO}_3$ per pot = 0.34 gm. N
4 N	4.12 gm. $\text{NaNO}_3$ per pot = 0.68 gm. N

These rates correspond approximately to field dressings of 0.25, 0.5 and 1.0 cwt. N per acre respectively, assuming that there are 1000 tons of soil per acre. Each of these three dressings was applied to different pots at seven different times throughout the growing season. There were thus twenty-one combinations of time of application and quantity of nitrogen. For each combination there were three replicate pots, and in addition there were nine control pots which received no nitrogen. The pots were placed on trucks in an unheated glasshouse. For the greater part of the growth period the trucks were pushed out into the open air, and were brought back under cover only in periods of severe frost or heavy rain. Watering was carried out with rain water collected from the roof of the glasshouse. The water content of the soil was maintained between 15 and 20 per cent. and was checked by weighing the pots at intervals. At no time was there any drainage from the pots, so that there was no loss of nitrogen by leaching.

The pots were arranged in three randomised blocks with twenty-four pots in each block—one for each of the twenty-one treatments and three controls.

*Experimental programme*

The wheat was sown on October 31, 1930, and the first seedlings appeared above the soil surface on November 10. By November 20 germination was complete. The number of plants per pot was then reduced to six.

The following table gives the times of application of the  $\text{NaNO}_3$  dressings and the corresponding stage of development of the plants:

Applica- tion	Date	Weeks after sowing	Stage of development
1	November 24, 1930	3	Immediately after germination
2	January 20, 1931	11	Tillering proceeding. Mean number of shoots per plant = 3
3	March 2, 1931	17	Mean number of shoots per plant = 7 (approximately half the maximum number)
4	March 30, 1931	21	Tillering almost ceased
5	April 27, 1931	25	Shoot number per plant reached its maximum
6	May 25, 1931	29	Late-formed tillers dying off. Ear-bearing shoots rapidly elongating
7	June 22, 1931	33	95 % of ears completely emerged from leaf-sheath

The pots were all harvested on August 11, 1931, 40 weeks after sowing.

*Observations made during growth and at harvest*

Counts of the number of living shoots on each plant and measurements of the height of the main shoot were made at intervals through the growing season. The height measurements were made to the auricle of the highest leaf until ear emergence, and later to the top of the ear. At harvest the number of ears per pot was counted. Ears were cut off at the basal notch, and ears and straw weighed separately. Subsequently the grain was rubbed out of the ear, the straw and chaff were dried in a steam oven and their dry weight recorded for each pot. The number of grains per pot was counted, and a dry-matter determination was carried out on a sample of grain from each pot. The dry weight of 1000 corns was calculated for each pot. Finally the grain from the three replicate pots of each treatment was pooled and a determination of total nitrogen content was carried out. Similarly straw and chaff were put together and the three replicates were pooled for a nitrogen determination.

## RESULTS

The results of the harvest data are presented in the form of tables giving the means of the three replicates of each treatment and of the nine controls. Each set of observations was analysed by the analysis of variance method, and the form of the analysis is shown in the example for total dry matter. In none of the analyses was the interaction between time of application and quantity of nitrogen found to be significant. That is to say, the effects of time of application were similar for the three levels of nitrogen application, and the relative response to the three rates of nitrogen application was not affected by time of application. In the discussion of the results, therefore, only the means for the seven times of application, for the three rates of nitrogen application, and for the untreated controls will be considered. In each table the standard errors are given of (a) the means for the seven times of application, and for the untreated pots, (b) the means for the three rates of nitrogen application, and (c) the difference between the means of all treated and of all untreated pots. Regressions on time of application were fitted for each variate, and the values of the regression coefficients which were found to be significant are given in the text. In the figures which show the effect of time of application, the magnitude of a significant difference, standard error (a)  $\times 3$ , is represented by a vertical line at the right-hand side of each graph, and the "no nitrogen" value of each variate is shown by a horizontal dotted line.

*Yield of total dry matter*

Table I gives the yields of total dry matter in gm. per pot. The fraction "No nitrogen *v.* nitrogen" in the analysis of variance represents the variance between the mean of all the treated pots and the mean of the controls. It is a measure of the average effect of nitrogen for all times and rates of application. *¶*

The mean increase in total dry matter produced by application of nitrogen was highly significant, but the difference in the response to the three rates of application was not significant. The effect of time of application was highly significant and its nature is shown in Fig. 1, where the mean yield of dry matter per pot is plotted against time of application, and the yield of the unmanured pots is also indicated. The yield of dry matter steadily decreased the later the nitrogen was applied, and the yield for the last time of application was not significantly different from that for the untreated pots. The linear regression coefficient of yield of total dry matter on time of application,  $-0.263 \pm 0.0646$  gm. per pot per week, was highly significant. The mean for the sixth time of application

was aberrant from the general trend, but its deviation from the regression line was not significant.

Table I. *Total dry matter (gm. per pot)*

Time of application	Nitrogen quantity			Mean
	1 <i>N</i>	2 <i>N</i>	4 <i>N</i>	
1	63.41	68.22	68.72	66.79
2	63.55	67.10	67.87	66.17
3	61.74	64.10	66.79	64.21
4	62.58	66.39	62.62	63.87
5	59.65	59.51	64.38	61.18
6	65.64	63.22	62.98	63.95
7	58.06	55.47	57.80	57.11
Mean	62.09	63.43	64.45	63.32
No nitrogen	—	—	—	58.49

S.E.: (a)  $\pm 1.652$ , (b)  $\pm 1.081$ , (c)  $\pm 1.766$ .

*Analysis of variance of yield of total dry matter*

	Degrees of freedom	Sum of squares	Mean square
Blocks	2	107.15	53.58
No nitrogen <i>v.</i> nitrogen	1	184.13	184.13
Quantity of nitrogen	2	58.96	29.48
Time of application	6	582.99	97.17
Interaction (nitrogen quantity $\times$ time of application)	12	162.70	13.56
Error	48	1179.26	24.57
Total	71	2275.19	—

*Yield of grain*

Analysis of the dry weight of grain per pot showed that there was a significant difference between the mean of the control pots and the mean of all the treated pots, but not between the means for the three rates of

Table II. *Dry weight of grain (gm. per pot)*

Time of application	Nitrogen quantity			Mean
	1 <i>N</i>	2 <i>N</i>	4 <i>N</i>	
1	23.60	25.83	25.04	24.82
2	23.85	24.48	25.32	24.55
3	22.89	23.44	24.42	23.58
4	22.77	25.34	24.38	24.16
5	23.41	22.99	25.96	24.12
6	26.62	25.00	26.24	25.95
7	22.39	21.06	19.51	20.99
Mean	23.65	24.02	24.41	24.03
No nitrogen	—	—	—	21.63

S.E.: (a)  $\pm 0.872$ , (b)  $\pm 0.571$ , (c)  $\pm 0.932$ .

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nitrogen application. Time of application had a highly significant effect which is shown in Fig. 1. The grain yields for the first six times of applications were not significantly different from each other, and, with the exception of that for time 3, all were significantly greater than the

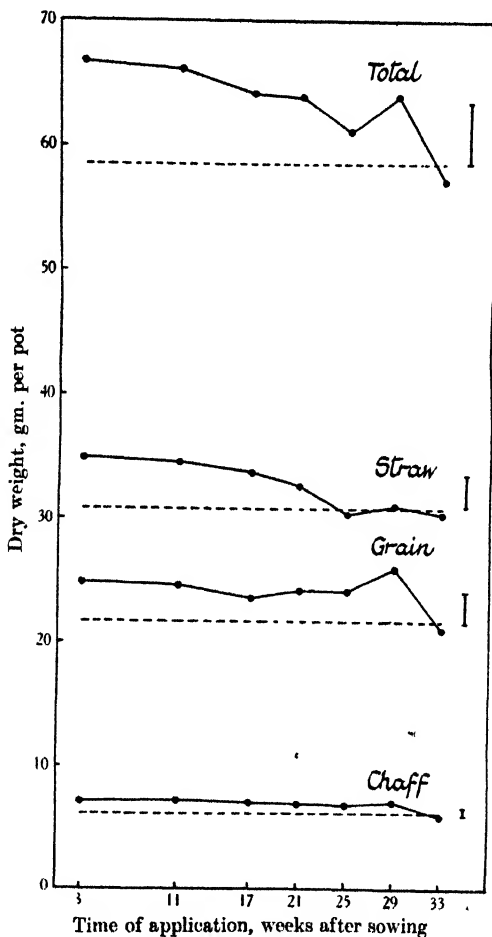


Fig. 1. For explanation see text.

control. The yield for the seventh time of application was significantly less than those for the first six times and did not differ from the control. Linear and quadratic regressions on time of application fitted to the first six times were not significant, and the results must, therefore, be interpreted as showing that applications of nitrogen at the first six times

produced equal increases in grain yield, while application at time 7 had no effect.

### *Yield of straw*

The difference in yield of straw between the means of the untreated and the treated pots just failed to reach significance, and the effect of nitrogen quantity was also not significant. The effect of time of application of nitrogen is shown in Fig. 1. The increased yield of straw due to nitrogen application decreased steadily from highly significant values at time 1 to small and insignificant values for times 5, 6 and 7, and the linear regression coefficient on time of application,  $-0.175 \pm 0.0327$  gm. per pot per week, was highly significant.

Table III. *Dry weight of straw (gm. per pot)*

Time of application	Nitrogen quantity			Mean
	1 N	2 N	4 N	
1	33.22	35.05	36.27	34.85
2	32.93	35.25	35.17	34.45
3	32.24	33.70	35.01	33.65
4	33.22	33.97	31.15	32.78
5	30.00	29.74	31.04	30.26
6	31.71	31.20	30.10	31.00
7	29.46	28.86	32.43	30.26
Mean	31.82	32.54	33.02	32.46
No nitrogen	—	—	—	30.75

S.E.: (a)  $\pm 0.837$ , (b)  $\pm 0.548$ , (c)  $\pm 0.895$ .

### *Yield of chaff*

The average effect of nitrogen application on the dry weight of chaff per pot was significant, but the difference between the mean yields for

Table IV. *Dry weight of chaff (gm. per pot)*

Time of application	Nitrogen quantity			Mean
	1 N	2 N	4 N	
1	6.59	7.35	7.42	7.12
2	6.78	7.37	7.38	7.17
3	6.61	6.96	7.37	6.98
4	6.59	7.08	7.09	6.92
5	6.24	6.79	7.38	6.80
6	7.31	7.03	6.64	6.99
7	6.21	5.52	5.86	5.86
Mean	6.62	6.87	7.02	6.83
No nitrogen	—	—	—	6.12

S.E.: (a)  $\pm 0.215$ , (b)  $\pm 0.140$ , (c)  $\pm 0.229$ .

Chaff here included all parts of the ear except the grain.

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the three rates of nitrogen application failed to reach significance. The effect of time of application (Fig. 1) was again highly significant, and was very similar to that found for grain yield. The yield was almost constant for the first six times of application of nitrogen, and significantly above the control, but the seventh application produced no increase.

#### *Analysis of the yield of grain*

Three variable attributes of growth together determine the yield of grain per plant, namely,

- (1) The weight of the individual grains (usually expressed as 1000-corn weight).
- (2) The number of grains per ear.
- (3) The number of ears per plant.

An increase in yield produced by manurial treatment may be due to an increase in any one of these factors, or to a simultaneous increase in two or three of them. The effect of the nitrate dressings and of the time of their application on these three aspects of grain yield will be considered next.

#### *1000-corn weight*

Table V gives the mean weights of 1000 dry corns for each treatment. The difference between the mean for the treated pots and the mean for the controls was not significant. The 1000-corn weight tended to decrease with increase in quantity of nitrogen applied, and there was a significant

Table V. *Dry weight of 1000 corns (gm.)*

Time of application	Nitrogen quantity			Mean
	1 N	2 N	4 N	
1	40.13	38.98	36.51	38.54
2	40.19	37.92	37.98	38.70
3	38.63	39.46	37.07	38.28
4	39.97	40.01	38.34	39.44
5	40.94	39.06	39.71	39.90
6	40.74	41.61	42.57	41.64
7	41.59	43.10	41.67	42.10
Mean	40.31	40.02	39.12	39.82
No nitrogen	—	—	—	39.30

S.E.: (a)  $\pm 0.500$ , (b)  $\pm 0.328$ , (c)  $\pm 0.535$ .

difference between the means for the largest and smallest rates of application. The relation between time of application of nitrogen and 1000-corn weight is shown in Fig. 2. The 1000-corn weights corresponding to the first three times of application were nearly equal, and were below the

values obtained for the untreated pots, though not significantly so. With later applications, the 1000-corn weight began to increase; the value for the fifth time of application was significantly greater than those for the first three times, and this increase continued for the later applications.

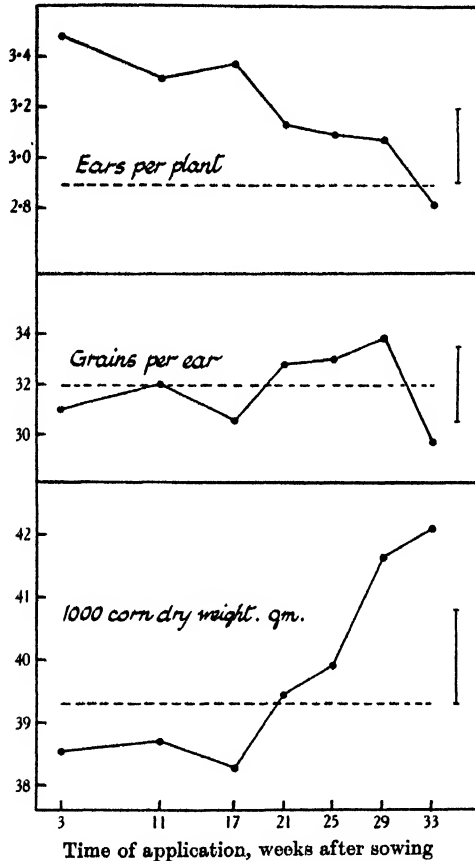


Fig. 2. For explanation see text.

The means for times 6 and 7 were significantly greater than the mean for the untreated pots.

A quadratic regression fitted to the seven values was highly significant, and accounted for 94 per cent. of the variance due to times of application. The regression equation was

$$y = 38.936 - 0.1314x + 0.00712x^2 \pm 0.361,$$

where  $x$  = time of application in weeks after sowing, and  $y$  = 1000-corn



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weight. The existence of this regression, and the absence of a significant difference between the mean for all times of application and the mean for untreated plants, is evidence that the depression of 1000-corn weight found for times 1, 2 and 3 was a real and not a chance effect.

### *Number of grains per ear*

The mean number of grains per ear was calculated for each pot by dividing the number of grains per pot by the number of ears per pot. The treatment means are given in Table VI. The analysis of variance showed no significant responses to treatment.

Table VI. *Number of grains per ear*

Time of application	Nitrogen quantity			Mean
	1 <i>N</i>	2 <i>N</i>	4 <i>N</i>	
1	30.61	30.15	32.17	30.98
2	30.78	31.31	33.85	31.98
3	31.43	28.20	31.98	30.54
4	32.74	34.08	31.82	32.78
5	34.98	32.73	31.17	32.96
6	34.01	31.84	35.67	33.84
7	30.16	31.91	27.06	29.71
Mean	32.11	31.46	31.96	31.84
No nitrogen	—	—	—	31.94

S.E.: (a)  $\pm 0.991$ , (b)  $\pm 0.648$ , (c)  $\pm 1.059$ .

The mean values for the seven times of nitrogen application and for the untreated pots are plotted in Fig. 2. The curve shows a tendency to rise to a maximum at time 6, but linear and quadratic regressions fitted to the seven values were not significant. The linear regression coefficient of number of grains per ear on time of application, expressed as weeks after date of sowing, fitted to the first six times, is  $0.1027 \pm 0.0465$  grain per ear per week, so that it is just significant. There is, therefore, some evidence that the number of grains per ear tended to increase with later application of nitrogen, when only the first six times are considered, and since, on the average of all times, nitrogen application produced no effect, this result must be interpreted as showing a depression for the early applications and an increase for the late applications. The mean for time 7 was significantly less than the mean of the first six times, but its difference from the mean of the untreated plants was not significant.

### *Number of ears per plant*

Table VII shows the mean number of ears per plant. The mean for all pots receiving nitrogen was significantly greater than the mean for the untreated pots, and the effect of time of application was also

highly significant (Fig. 2). The earlier the application of nitrogen the greater was the number of ears produced per plant. The first three applications gave significant increases above the value for the untreated plants, which were not significantly different from each other. The linear regression coefficient of ear number on time of applications,  $-0.0200 \pm 0.00837$  ear per plant per week, was highly significant, and the goodness of fit of the regression suggests that the increases produced by the fourth, fifth and sixth treatments were real, although these increases were not individually significant. The seventh application did not affect the number of ears per plant.

Table VII. *Number of ears per plant*

Time of application	Nitrogen quantity			Mean
	1 N	2 N	4 N	
1	3.22	3.67	3.56	3.48
2	3.22	3.44	3.28	3.31
3	3.17	3.50	3.44	3.37
4	2.94	3.11	3.33	3.13
5	2.72	3.06	3.50	3.09
6	3.22	3.33	2.89	3.07
7	3.00	2.56	2.89	2.81
Mean	3.07	3.21	3.27	3.18
No nitrogen	—	—	—	2.89

S.E.: (a)  $\pm 0.099$ , (b)  $\pm 0.065$ , (c)  $\pm 0.106$ .

The results of this analysis show that although the increase in yield of grain produced by the addition of sodium nitrate was almost constant for the first six times of application, the method by which the yield increase was produced varied with the time of application of nitrogen. The constancy of yield was the result of opposite trends in the number of ears per plant on the one hand and the number of grains per ear and the 1000-corn weight on the other. Thus, early applications caused a large increase in the number of ears per plant, and small decreases in 1000-corn weight and the number of grains per ear, while late applications caused only a small increase in the number of ears per plant, but also increased the 1000-corn weight and the number of grains per ear.

Application of nitrogen at time 7 did not increase the grain yield, but produced a qualitative effect. The 1000-corn weight was increased, but this was offset by small, and not significant, decreases in number of grains per ear and number of ears per plant.

*Number of shoots per plant*

Counts of the number of living shoots per plant were made on nine occasions during the growth period. Each set of counts was examined by the analysis of variance method, in a similar manner to the yield data. The form of the analysis differed slightly for the earlier counts, since these were made before the later applications of nitrogen. The number of pots on which the "no nitrogen" mean is based, changed throughout the course of the experiment as more treatments were applied, and this must be borne in mind in using the standard errors.

The interaction between time of application and quantity of nitrogen was not significant in any of the sets of counts, and all the information obtainable from the results is given by the means of all quantities of nitrogen for each time of application, and the means of all times for each quantity of nitrogen. These are set out in Table VIII, together with the means for the untreated plants, and the appropriate standard errors are given.

The number of shoots per plant increased, at first rapidly and then more slowly, to reach a maximum at the end of April. Later there was a rapid death of shoots, and at the end of June only those shoots survived which formed ears.

No significant effects on shoot number were found until March 19. On this and all subsequent dates, the mean for all treated plants was significantly greater than the mean for untreated plants. In the counts previous to May 23, the means for the three nitrogen quantities did not differ significantly. On this date the means for the 1 *N* and 2 *N* quantities were both significantly less than that for 4 *N*. Later 1 *N* was significantly less than 2 *N* and 4 *N*, which were nearly equal. It appears that during the period when the number of shoots per plant was rapidly increasing, the three rates of nitrate application were equally effective in increasing the number of shoots per plant. A difference between the three rates of application appeared only in the subsequent phase of growth when the late-formed tillers were dying off, and in this phase the number of shoots per plant which survived increased with increase in the amount of nitrogen applied.

The effect of time of application of nitrogen on the mean number of shoots per plant is shown in Fig. 3. The curve for the untreated pots is given in the lower half of the figure to show the course of tillering, and in the upper half the differences between the means for each time of application and the mean for untreated plants are plotted on a larger scale.

Table VIII. *Mean number of shoots per plant*

Date	Time of application (mean of all rates)						Linear regression coefficient	S.E.	No. nitro-gen	S.E.	Nitrogen quantity (mean of all times)				S.E.	Mean of all nitro-gen appli-cations	S.E.
	1	2	3	4	5	6					1 N	2 N	4 N	4 N			
Jan. 7	2.09	—	—	—	—	—	—	±0.072	2.07	±0.027	2.00	2.28	2.00	2.00	±0.121	2.09	±0.072
Jan. 21	3.11	2.96	—	—	—	—	—	±0.085	3.02	±0.035	2.94	3.14	3.02	3.02	±0.105	3.02	±0.060
Feb. 28	7.50	7.15	—	—	—	—	—	±0.237	6.96	±0.097	7.22	7.72	7.03	7.03	±0.290	7.32	±0.168
March 19	13.52	12.98	12.56	—	—	—	—	±0.397	12.08	±0.177	13.17	13.19	12.70	12.70	±0.397	13.02	±0.229
April 9	14.52	13.94	13.41	13.07	—	—	—	±0.393	12.78	±0.197	13.79	13.88	13.54	13.54	±0.341	13.74	±0.197
April 26	14.70	14.06	13.56	13.11	—	—	—	±0.397	12.79	±0.199	13.78	13.97	13.81	13.81	±0.344	13.86	±0.199
May 23	10.06	10.13	9.83	9.65	9.80	—	—	±0.317	8.41	±0.183	9.29	9.82	10.57	10.57	±0.245	9.89	±0.142
June 11	3.65	3.57	3.59	3.33	3.39	3.37	—	±0.106	3.06	±0.075	3.26	3.57	3.62	3.62	±0.075	3.48	±0.043
June 22	3.50	3.37	3.41	3.19	3.13	3.13	—	±0.101	2.89	±0.072	3.10	3.38	3.38	3.38	±0.041	3.29	±0.024

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Owing to the fact that there were more replicates of the controls than of the times of application, different standard errors are appropriate for the comparison of two times of application, and of one time of application with the control. In Fig. 3, significant differences corresponding to

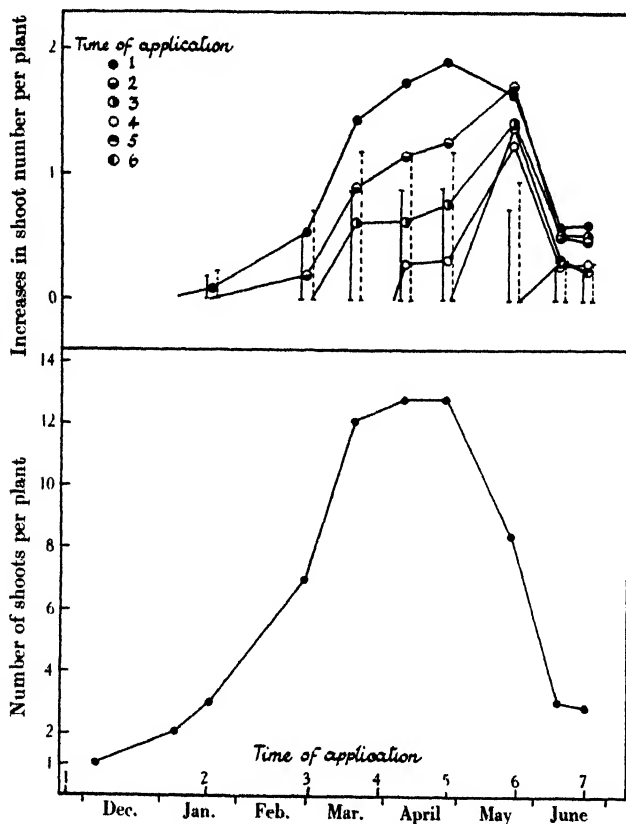


Fig. 3. The lower half of the figure shows the mean number of shoots per plant, for the untreated plants. The upper half shows the mean increases in shoot number per plant produced by nitrogen application at different times. The full vertical lines indicate significant differences for testing the deviation of the mean increases for individual times of application from zero, and the vertical dotted lines represent significant differences between mean increases for any two times of application.

these two standard errors are represented by vertical lines at each observation time. Significant differences between times of application were found in the analysis of variance for April 26 and June 22 but not for other occasions, and the nature of the effects becomes clear when the curves in Fig. 3 are considered.

Up to the time of maximum shoot number at the end of April, the earlier the time of application of nitrogen the greater was the number of shoots per plant. That is to say, tillering was greatest in those plants which received nitrogen immediately after germination, and though applications 2, 3 and 4 tended to cause an increase in tillering, the plants receiving these later dressings never had as many shoots as plants which received the earliest dressing. Although there was no significant difference between times of application on March 19 and April 9, only times 1 and 2 gave significantly greater shoot numbers than the untreated controls. On April 26 the shoot number corresponding to time of application 1 was significantly greater than that corresponding to time 4. Applications 2 and 3 were intermediate between 1 and 4, and not significantly different from each other or from 1 or 4. Times 1 and 2 both gave significantly greater shoot numbers than the control, but 3 and 4 did not differ significantly from the controls. Significant negative linear regression coefficients of shoot number per plant on time of application of nitrogen were found on April 9 and 26.

The highest shoot numbers were found on April 26 for all treatments, and the nitrogen applications do not appear to have affected the time of maximum shoot number. In the subsequent phase of dying off of the late-formed tillers, the differences between times of application disappeared. On May 23 the mean shoot numbers per plant for the first five times of application were almost equal, and all were significantly above the untreated control. Application 5, made only 4 weeks previously, produced almost as great an increase of shoot number as the earliest application. The regression of shoot number on time of application of nitrogen was not significant.

On June 11, all nitrogen dressings previously applied produced a significant increase of mean shoot number per plant, including the application at time 6, made only 17 days previously. Although the analysis of variance showed no significant differences between times of application, the linear regression coefficient of shoot number on time of application of nitrogen was again significantly negative, showing that the differences between times of application, which were absent on May 23, were beginning to appear again.

This tendency of the curves for the successive times of application to separate persisted, and on June 22, when practically all shoots not bearing ears had died, the means for times 1, 2 and 3 were all significantly greater than the means for times 5 and 6, and the mean for time 1 was significantly greater than that for time 4. Times 1, 2, 3 and 4 all gave

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significantly greater shoot numbers than the untreated control. The increases due to applications at times 5 and 6 were equal, but just failed to reach significance. The linear regression coefficient of shoot number on time of application of nitrogen was highly significant. The last application at time 7 had no effect on shoot number, since it was made at a time when only ear-bearing shoots survived.

In general, the earlier the application of nitrogen the greater was the increase in shoot number produced. The difference between the effects of different times of application was least marked at the stage of rapid death of the youngest shoots. At this stage also, differences in shoot number between the three rates of application of nitrogen first appeared. This dependence of surviving shoot number on the amount of nitrogen available to the plant, irrespective of when it was applied, suggests that there was at this time an intense competition between shoots for nutrients, due to their rapid increase in size.

The differences between treatments in straw yield were almost entirely accounted for by differences in shoot number at harvest, since the weight of straw per ear-bearing shoot (Table IX) varied little and showed no significant treatment effects.

Table IX. *Mean weight of straw in gm. per ear-bearing shoot*

Time of application							No nitrogen	S.E.
1	2	3	4	5	6	7		
1.67	1.73	1.67	1.76	1.65	1.69	1.81	1.79	$\pm 0.044$
Nitrogen quantity							S.E.	
1 N		2 N		4 N				
1.74		1.70		1.69			$\pm 0.029$	

The maximum shoot number per plant, fourteen, was considerably higher than the mean value for a field crop, which is usually not greater than four. This indicates that the conditions of growth, probably of spacing in particular, were more favourable, and the competition between plants less intense, than in a field crop. At the time of maximum shoot number, elongation of the shoots has only just begun; the plants are small and the shading effect of one plant on another is likely to be slight, and the most important factor in competition is probably the available nutrient supply. In conditions of more severe competition, induced for example by closer spacing, it is therefore probable that the effects of an increased supply of nutrients would be more marked, and it is improbable

that the results recorded here are specific to conditions which induce a high rate of tillering.

### *Height of main shoot*

The mean height of the main shoot corresponding to the different treatments is shown in Table X.

At no time during the course of the experiment was a significant effect of time of application on shoot height observed. In April and May the mean for all treated plants was greater than that of the untreated plants. At the end of May this difference disappeared, and subsequently a significant depression of the height of the main shoot by nitrogen application was recorded. In the measurements made on June 22 and August 10, there was some evidence that the depression increased with increasing quantity of nitrogen applied. The depression was associated with an increase in the number of shoots per plant, and was probably due to intershoot competition.

### *Nitrogen content at harvest*

The results of the determinations of total nitrogen content carried out on the harvest samples of grain and straw and chaff are given in Table XI and Fig. 4.

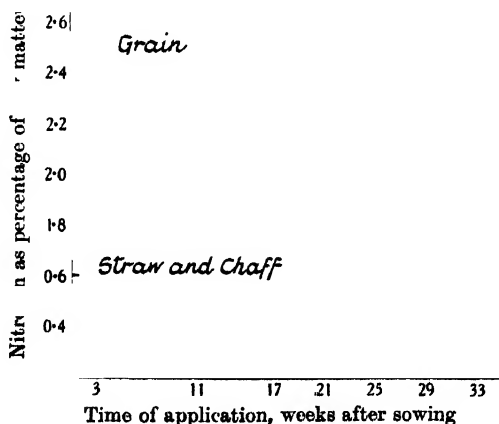


Fig. 4. For explanation see text.

Owing to the bulking of replicates before the analyses were made, no direct estimate of error was possible. The fraction of the total variance attributable to the interaction between times and rates of application was, therefore, used as the error term in the analysis of variance with



Table XI. Nitrogen content at harvest

[illegible]

which to compare the variances due to times and rates of application. The standard errors given in Table XI were calculated on this basis. The absence of any significant interaction in the analyses of the yield data justifies this procedure and suggests that it does not involve a serious overestimation of error.

The nitrogen content of the grain, expressed as a percentage of dry matter, was significantly increased by application of nitrogen at all times, and there were significant differences between times of application. There was little difference between the first three times, but with later applica-

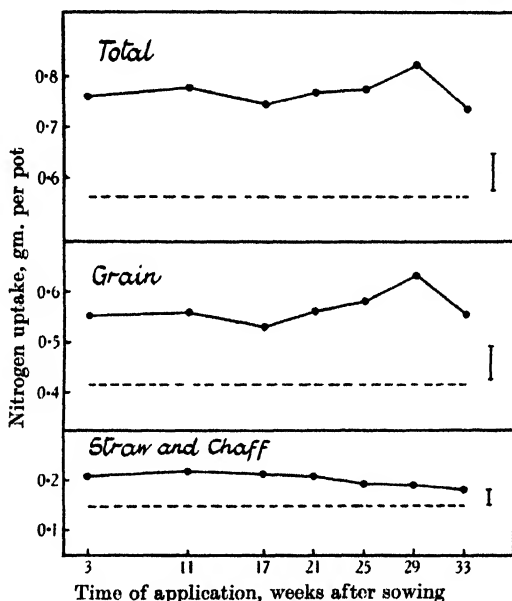


Fig. 5. For explanation see text.

tions there was a steady rise of nitrogen content. A quadratic regression fitted to the seven values was highly significant and accounted for 90 per cent. of the variance. The regression equation was

$$y = 2.273 - 0.0128x + 0.000704x^2 \pm 0.0475,$$

where  $x$  = time of application in weeks after sowing, and  $y$  = nitrogen as percentage of dry matter. The differences between the means for the three rates of application were also significant.

The percentage of nitrogen in straw and chaff was significantly increased by nitrogen application at all times, but the differences between times were small and not significant, and no significant regression on time

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of application was found. The differences between the means for the three rates of application were significant.

Table XI and Fig. 5 show the nitrogen uptake in gm. per pot in grain, in straw and chaff, and in grain, straw and chaff together. In both grain and straw and chaff the nitrogen uptake was significantly increased by application of nitrogen, but the differences between times of application were not significant.

The nitrogen uptake in the grain showed some tendency to rise to a maximum at time 6, falling again at time 7. Linear and quadratic regressions on time of application were not significant, but omitting time 7 the linear regression coefficient was  $0.00242 \pm 0.00104$ , and was significant. This result may be interpreted as an indication, but not a conclusive demonstration, that the nitrogen uptake in the grain increased with later application of nitrogen for the first six times of application, and this agrees with the constancy of grain yield for these times combined with the steady increase of nitrogen content as percentage of dry matter with later application, which have been shown previously.

The nitrogen uptake in straw and chaff showed a significant negative linear regression,  $-0.00107 \pm 0.00039$  gm. N per week, on time of nitrogen application. The drifts of nitrogen uptake in grain and in straw and chaff with time of application were opposite in sign, and no significant regression of the nitrogen uptake in grain, straw and chaff combined on time of nitrogen application could be demonstrated. These results are interpreted as showing that the total nitrogen uptake was equal for all times of application, but that the distribution of nitrogen uptake between grain and straw and chaff varied with the time of application, in the sense that the later the time of application the higher was the fraction of the total nitrogen uptake found in the grain and the lower the fraction found in straw and chaff. This is made clear if the ratio of nitrogen uptake in grain to nitrogen uptake in straw and chaff is examined. The values of the ratio are given in Table XI. Though differences between the ratios for individual times of application were not significant, a significant linear regression was found. The value of the regression coefficient was  $0.0248 \pm 0.00775$ .

There were no significant differences in the percentage recovery of added nitrogen, either between times of application or between rates. The average recovery was 51.0 per cent.

*Ripening*

During the period of ripening of the plants at the end of July, and in early August, it was very obvious that late nitrogen applications, particularly at time 7, delayed the drying out of the plants and the change of colour from green to yellow. Although harvest was delayed to allow all plants to become fully ripe, significant differences between times of application were found at harvest in the dry-matter content expressed as percentage of fresh weight of the straw. This is shown in Table XII.

Table XII. *Dry matter as percentage of fresh weight*

	Time of application (mean of all rates)							No nitrogen	S.E.	Nitrogen quantity (mean of all times)				S.E.
	1	2	3	4	5	6	7			1 N	2 N	4 N		
Straw	74.8	72.5	71.8	71.5	73.6	70.2	60.0	66.3	±1.93	68.9	70.2	72.8	±1.26	
Ears	84.8	83.7	85.0	83.5	83.5	83.2	82.4	82.8	±0.64	83.9	83.6	83.6	±0.42	

The dry-matter content of the straw for the mean of all treated plants was significantly greater than the mean for the untreated controls, but this effect of nitrogen was found only at the first six times of application.

The dry-matter content of the straw for time 7 was significantly less than that for the other times of application, which did not differ significantly from each other.

There were no significant differences between treatments in the dry-matter content of the ears. The dry-matter content of the grain at harvest was not determined, as the ears were partially dried before the grain was separated.

## DISCUSSION

The results of this experiment show that the ability of wheat to take up nitrogen applied to the soil does not vary with the time at which the nitrogen is applied, for the nitrogen recovery was constant, within the limits of error, for all times of application. The effects of varying the time of application cannot, therefore, be attributed to differences in total nitrogen uptake.

The steady fall of the increase in total dry-matter production with later applications is readily explained physiologically. The increase in dry matter must be attributed to increased leaf area, since Gregory (4) has shown that varying nitrogen supply does not affect the rate of assimilation per unit leaf area. The increase of leaf area may be produced either by an increase in the number of leaves, through the formation of more shoots, or

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by an increase in the size of individual leaves. The earlier the nitrogen application is made, the earlier must the increased leaf area be developed, so that the rate of assimilation per plant must be increased over a larger period of time, and consequently the increase in total dry matter at harvest is greater. It has been shown that early application caused a greater increase in shoot number, and therefore presumably of leaf number, than late, and unless late application compensated for this by causing a greater increase in leaf size, it may account in part for the greater efficiency of early application in increasing the total dry-matter production.

Straw yield varied with time of application in a similar manner to the total dry matter, and it has been shown that the variation was attributable to changes in shoot number. Grain yield, however, showed quite different effects, for which it is not easy to find an explanation. As three rates of application of nitrogen were used in the experiment, the increase of grain yield for the mean of the three rates might have been constant for the first six times of application by chance, if the trend of grain yield varied with the rate of application, but Table II shows that this explanation is not possible. It may be supposed that the amount of carbohydrate translocated to the grain is dependent not only on the total amount of carbohydrate available, but also on the amount of nitrogen. With later applications there is a greater quantity of mobilisable nitrogen in the plant which can be translocated to the grain, as a smaller quantity is locked up in the permanent structure of the straw, and this may enable a larger fraction of the total carbohydrate material to be withdrawn from the shoots by the developing grain.

The manner in which the increase of grain yield was produced varied with the time of application, depending on the potentialities of growth in the subsequent period. Doughty and Engledow (2), in a study of the growth of a field crop of wheat, observed that shoots produced later than the end of March did not form ears, and have postulated that at this period there is a "critical time" for shoot formation. They suppose that "A dressing of nitrogenous fertiliser applied after the critical date . . . , may promote the formation of tillers too late to bear ears. It may, nevertheless, valuably stimulate the earlier existing tillers, allowing them to bear heavier ears than otherwise they would." There is no evidence of the existence of such a critical date in the data of the present experiment. The relation between the increase in the number of ears per plant at harvest and the time of nitrogen application was well fitted by a linear regression which indicated a zero increase at about the time of ear

emergence, and there was no suggestion of a sharp extinction of the effect on ear number at an earlier date. Reference to Fig. 3 shows that all shoots which ultimately formed ears had been produced by January 20, yet an application of nitrogen 6 weeks later, on March 3, caused a significant increase in the number of ears per plant at harvest, and even later applications, on March 30, April 27 and May 25, caused small increases in the number of ears, which though not individually significant, were probably real, since they fitted closely to the regression line.

Similarly, the relations of number of grains per ear and 1000-corn weight to time of nitrogen application showed no evidence of a critical date, but were well fitted by smooth curves indicating a continuity of effect. The general hypothesis<sup>(5)</sup> that early applications increase grain yield mainly by increasing the number of ears, while late applications tend to increase the size of the ear through both number and size of grains, was, however, confirmed.

The bearing of these results on the practical problem of the manuring of field crops of wheat is obvious. Ignoring for the moment the influence of seasonal weather conditions, it appears that a rather late application in May, corresponding to time 6, is likely to be most efficient. Application at that time produced the same increase in grain yield as earlier applications, but a smaller increase in straw yield, so that late application to a field crop is less likely to increase its susceptibility to lodging than early application. Late application also improved the quality of the grain, increasing its size and nitrogen content. This agrees with the work of Gericke<sup>(3)</sup>, who found that the protein content of the grain was higher the later nitrogen was applied, and of Davidson and Le Clerc<sup>(1)</sup>, who showed that application of nitrogen even as late as the time of ear emergence raised the protein content.

The extent to which these conclusions must be modified, in relation to seasonal weather conditions, can only be determined by direct experimentation in the field, but it appears that they will hold true, unless weather conditions, and particularly insufficient rainfall, subsequent to a May application, prevent a full uptake and utilisation of the nitrogen applied.

#### SUMMARY

The effect of applying a nitrogenous fertiliser to wheat at seven different times and three rates, was studied in pot culture. It was found that the later the time of application the smaller was the increase in the yield of total dry matter and of straw. The increase in the yield of grain,

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on the other hand, was constant for the first six times of application, the last of which was made on May 25, but a later application made after ear emergence produced no increase.

Analysis of the grain yield showed that early application produced its effect by increasing the number of ears per plant. Later applications caused a smaller increase in ear number, but also increased the number of grains per ear and 1000-corn weight. There was no evidence of a critical time for tiller formation such as has been postulated by Doughty and Engledow.

The increase in total nitrogen uptake was equal for all times of application, but the ratio of nitrogen in grain to nitrogen in straw and chaff was greater, the later the time of application.

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# INVESTIGATIONS IN CROP HUSBANDRY

## IV. THE PREPARATION OF THE SEED BED FOR WHEAT AFTER POTATOES

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### INTRODUCTION

ALTHOUGH cultivations are fundamental to arable farming they have received but little attention from the scientific investigator. Unless science is prepared to accept as inevitable that cultivation should remain purely an art, some attempt must be made to compare, from the point of view of crop yield, the common operations of tillage. The difficulties encumbering such investigations are only too clear. Conclusions reached in one season on a particular soil are, by themselves, entirely valueless and there can be no doubt that before the whole subject of tillage can even begin to assume scientific shape a vast amount of experimentation must be undertaken. It may be forecast that gradually a science will be evolved, but at the present time workers have practically a clear field before them. The field must be covered piecemeal, and the present paper claims to turn only a very diminutive furrow.

In this country main-crop potatoes are usually followed by wheat, one of the reasons responsible for this sequence being that the work done in connection with lifting the potatoes leaves the land almost ready for wheat. This is evidenced by the fact that wheat is not infrequently drilled directly after the final lifting operation, which is harrowing to collect tops and to expose covered tubers. This method of procedure can only be followed in a dry autumn because if the potatoes are lifted during wet weather the land is left in a solid condition; in most of such cases it is only necessary to stir the soil once or twice with a cultivator to loosen it, and thus to facilitate the penetration of the drill coulters. On the heavier loams, and especially in wet seasons, a more thorough preparation may be necessary, and then the sequence is sometimes the plough followed by a light harrow to level the surface. This method, though more expensive, has two advantages over and above the loosening it gives—weeds that have survived the potato-lifting operations are buried, and some clod is



brought to the surface to give protection and to reduce the chance of surface capping. All these methods are in common usage in this country, and it was decided to embark on a series of experiments to compare them, not only for the direct practical importance of the question but also because it was thought that some light might be thrown on the kind of seed bed required by autumn-sown cereals.

#### DESCRIPTION OF EXPERIMENTS

On the University Farm, Cambridge, only main-crop potatoes are grown, and they are restricted to the lighter fields which extend to some 80 acres. This land is not typical of the potato soils of the country; it is irregular in depth, and though in general a light gravelly soil it contains an appreciable, and very variable, amount of clay. In every case the potatoes had been grown as a normal farm crop, the areas not being taken for experiment until after the potatoes had been removed; the wheat experiments were all on a field scale, normal tillage implements being used. In every case the variety of wheat was Little Joss, which has established itself as the most satisfactory for the light land of the University Farm. In the years 1929–30 and 1930–1 the experiment took the form of a  $4 \times 4$  Latin square with split plots. There were four main treatments in the experiment, and the main plots were split for a manurial enquiry; the main treatments were ploughing and cultivating for the seed bed, each with and without spring cultivations. None of the interactions between seed-bed preparations on the one hand, and spring cultivations or manurial treatments on the other, were significant, and consequently no further reference will be made to the last two. Thus in each of these two experiments there were eight effective replications for the comparison of the plough and the cultivator. In the years 1931–2 and 1932–3 the experiments consisted of randomised blocks with split plots. The main plots were devoted to a comparison of four spring treatments, and each main plot was divided into three, over which were randomised three seed-bed treatments—ploughing, cultivating and nothing (*i.e.* direct drilling); in 1931–2 there were seven blocks and in 1932–3 there were six, so that the numbers of replications for the seed-bed comparisons were 28 and 24 respectively. Large discards had to be allowed for turning the implements; in the case of the Latin squares these were obtained by spacing the columns, and in the case of the randomised blocks by spacing the blocks.

For developmental studies random foot lengths were taken. In the first year these were distributed all over the plot, but the extreme lodging

of the crop precluded their extraction at harvest. Because of the trampling involved in making counts during growth the samples were restricted in the last three years to a yard across the end of each plot (in which yard they were randomised). This method of restricting the samples to the ends of plots is far from satisfactory, as no fair representation of the plots is obtained; the method was, in fact, abandoned for the harvesting of the crop in the last year, when the samples were dispersed all over the plots. The samples were used for all developmental counts and to determine ear number and (by covariance) ear size; samples having been removed the whole plots were cut to give figures for yield of grain and straw. In the first three years it was planned to cut the plots with a binder, though lodging prevented this in 1929-30; in view of the large discards that had to be cut before the binder could be introduced, and of the desirability of obtaining neater sheaves, it was decided in the last year to cut the whole plots by sickle, and this method has proved preferable.

The results of a cultivation experiment must depend very largely upon the soil conditions at the time the cultivations are carried out, and consequently some attention must be devoted to the conditions in these four experiments. The summer of 1929 was abnormally hot and dry, and the drought continued well into October. After the potatoes had been lifted the top 4-5 in. of the soil were in an extremely fine, dry condition, whereas the unworked soil beneath was exceedingly firm. In these circumstances the cultivator tines had little or no effect; the ploughing did very little more, because the loose top soil was merely pushed to one side and did not hold the implement sufficiently to prevent its riding over the firm soil beneath. At no time was there any appreciable difference between the appearance of the soil on the two series of plots; nor did they differ in firmness.

The conditions in the summer of 1930 were very different; July, August and September were wet months, but the October rainfall was only half the normal for the month. When the potatoes had been removed the land was left in fairly good condition. The surface was fine and fairly firm and the cultivator loosened it appreciably; the plough brought a considerable amount of fair-sized clod to the surface, and the relative roughness of the ploughed plots showed up throughout the winter and spring. A little difficulty was experienced in maintaining a constant depth of sowing over the whole experiment, and it is probable that the depth averaged half an inch more on the ploughed, than on the cultivated, plots.

The months July-October in 1931 were very similar as regards rainfall

to those of 1930, producing similar conditions after the potatoes had been lifted. In 1931 the third treatment (neither cultivated nor ploughed) was introduced, and on that series of plots the surface was so firm that it was difficult to get the drill coulters into the ground at all; consequently the depredations of birds caused considerable loss of "plant" on those plots. The lightening action of the cultivator was sufficient to let the drill coulters in, making the average depth of drilling approximately  $1\frac{1}{2}$  in.; this gave adequate cover and thus protection from birds. Again the plough gave a cloddy surface; it also left the land very loose, with the result that the depth of sowing averaged at least  $2\frac{1}{2}$  in. Every effort was made to drill at a constant depth over the whole series, but the full application of the press was insufficient to cause penetration on the untouched plots, whilst the coulters sank deeply into the ploughed plots when no press was applied. It is felt that the variation in depth of sowing was inseparable from the treatments: had the three treatments been applied to three separate fields, as in practice, differences in depth of seeding could not have been avoided. This difference in seed depth was unfortunate, but with the adequate discards allowed between plots it cannot be ascribed to the experimental technique and should be regarded as an inevitable concomitant of the treatments. This year was differentiated from the other three in that many annual weeds (chiefly *Stellaria media* and *Veronica* spp.) survived the potato lifting, and the untouched plots were rather foul in the early stages of the wheat crop; the plough killed and buried these weeds very efficiently, whilst the disturbance caused by the cultivator killed a large proportion.

The summer of 1932 was not so wet as that of the preceding years, but in October the rainfall was very heavy, delaying the potato lifting by nearly a month. The unfavourable conditions at the time of lifting led to serious poaching of the land, and this was especially bad on the two experimental blocks on the heavier soil. The plots that were neither ploughed nor cultivated were very "sad", and again the drill coulters did not penetrate sufficiently to give a cover to the seed. The cultivator did not penetrate to more than  $1-1\frac{1}{2}$  in., and it produced no stirring effect; the plots were cultivated twice, but the depth of drilling was little more than that on the untouched plots. After ploughing the surface was very rough, and even two passages of the heavy drag harrows failed to produce much tilth; here the depth of drilling was undoubtedly greater, but some "plant" was lost through the roughness of the seed bed.

The agricultural details of the experiments are summarised in Table I.

Table I. *Agricultural details of experiments*

Year	Date	Soil type	Size of plot acres	Mean yield		Remarks
				Grain bushels/acre	Straw cwt./acre	
1929-30	Oct. 23	Light	1/16	27.75	65.50	Seed bed very fine and dry. Crop laid flat
1930-31	Oct. 20	Loam	1/16	45.60	32.51	Ploughed plots showed rough throughout winter. Some lodging and "foot-rot" at harvest
1931-32	Nov. 3	Light	1/40	35.09	53.73	Untouched plots characterised by great firmness at drilling, loss of seed by birds, greater prevalence of <i>Stellaria media</i> in spring. Some lodging at harvest
1932-33	Nov. 18	Four blocks loam and two heavy	1/48	35.04	30.03	Potatoes lifted in wet weather and seed bed rather rough, especially after ploughing. Crop light, but stood well and had no disease

The numerical results for developmental studies and for harvest yields are given in Table II.

The differences in conditions at drilling time, discussed above, explain the apparently variable results as regards germination. In the first and last years there were no significant differences between the treatments; that there should have been no differences in 1932-3 is rather surprising in view of the marked differences in drilling depths. In the second year the roughness of the ploughed plots led to a rather thinner "plant", whilst in the third year the shallow sowing on the untouched plots led to a loss of 13 per cent. by birds.

The second section of Table II suggests that the less the depth of sowing the more early tillers were produced. In all four cases (three being significant) the cultivated plots carried a greater number of stems at that count. The low position taken by the untouched plots in 1931-2 was due to the poor "plant" left by the birds. The position remained much the same with the late stem counts and these numerical measures of the growth of the crop were in agreement with general observations.

The autumn of 1929 was mild and favoured growth, so that the crop rapidly established itself and became definitely "winter proud"; it was impossible, however, to correct this, either by cutting or grazing, because the months of March, April and May were abnormally wet. Some

Table II. *Results of experiments*

Year	Date	Treatment			S.E. of relative mean	Significance
		Ploughed	Culti- vated	Nothing		
Germination count						
1929-30	Dec. 9	100.0	94.8	—	2.89	Insignificant
1930-31	Dec. 5	100.0	107.6	—	1.16	Cultivated > Ploughed ( $P < 0.01$ )
1931-32	Dec. 7	100.0	98.9	87.1	1.60	Ploughed and Cultivated > Nothing ( $P < 0.01$ )
1932-33	Jan. 17	100.0	104.7	105.6	3.22	Insignificant
Early tiller count						
1929-30	Feb. 18	100.0	102.1	—	1.69	Insignificant
1930-31	Feb. 24	100.0	132.6	—	2.55	Cultivated > Ploughed ( $P < 0.01$ )
1931-32	Feb. 20	100.0	107.3	92.6	2.10	Cultivated > Ploughed ( $P < 0.05$ ) Ploughed > Nothing ( $P < 0.05$ )
1932-33	Mar. 10	100.0	110.6	109.5	1.26	Cultivated and Nothing > Ploughed ( $P < 0.05$ )
Late tiller count						
1929-30	Apr. 15	100.0	98.5	—	1.80	Insignificant
1930-31	Apr. 8	100.0	127.3	—	3.63	Cultivated > Ploughed ( $P < 0.01$ )
1931-32	Apr. 11	100.0	103.1	97.4	1.82	Insignificant
1932-33	Apr. 8	100.0	111.5	116.7	3.79	Nothing > Ploughed ( $P < 0.01$ ) Cultivated > Ploughed ( $P < 0.05$ )
Ear number						
1929-30	Harvest	—	—	—	—	—
1930-31	"	100.0	98.1	—	2.58	Insignificant
1931-32	"	100.0	102.4	100.3	1.86	Insignificant
1932-33	"	100.0	106.3	107.3	2.56	Cultivated and Nothing > Ploughed ( $P < 0.05$ )
Corrected ear weight						
1929-30	Harvest	—	—	—	—	—
1930-31	"	100.0	89.8	—	4.01	Insignificant
1931-32	"	100.0	98.6	100.6	1.44	Insignificant
1932-33	"	100.0	94.3	94.2	1.85	Ploughed > Cultivated and Nothing ( $P < 0.05$ )
Yield of grain						
1929-30	Harvest	100.0	99.7	—	3.47	Insignificant
1930-31	"	100.0	86.5	—	3.21	Ploughed > Cultivated ( $P < 0.05$ )
1931-32	"	100.0	104.4	105.2	1.83	Cultivated and Nothing > Ploughed ( $P < 0.05$ )
1932-33	"	100.0	97.8	96.1	2.19	Insignificant
Yield of straw						
1929-30	Harvest	100.0	93.3	—	3.10	Insignificant
1930-31	"	100.0	89.0	—	1.78	Ploughed > Cultivated ( $P < 0.01$ )
1931-32	"	100.0	98.8	99.7	1.83	Insignificant
1932-33	"	100.0	98.9	96.7	2.88	Insignificant

lodging occurred on both series of plots in early May and no recovery ensued; a very severe thunderstorm in mid-June laid the whole crop flat, in which position it remained till harvest. Misfortune dogged this experiment; no appreciable difference in seed bed was obtained between

treatments, and the extreme lodging was sufficient to obliterate any small differences at harvest.

The growth of the 1930-1 crop was more normal. In that case a marked difference in seed bed was obtained, and the greater roughness of the ploughed plots was accompanied by a more robust plant growth; it was observed in spring that the wheat on the ploughed plots had a more upright habit of growth and a definitely darker green colour. In this case the wheat was not too forward, but nevertheless some lodging occurred shortly before harvest; the experimental crop also suffered slightly from "foot-rot". Immediately prior to cutting, the percentage of the crop laid and the percentage diseased were estimated for each plot; statistical analysis of these series of figures failed to reveal any significant difference between the ploughed and the cultivated plots.

The comparison of ploughing and cultivating proceeded very similarly in the following year. Again the ploughed plots appeared much stronger in spring, whilst the untouched plots carried plants which appeared definitely subnormal in growth; the table shows that the untouched plots carried only 3 per cent. less stems than the ploughed, but extensive weighings of lifted plants showed that the dry weight of the whole plants was 25 per cent. less. Both the late-stem count and the dry-weight determinations failed to bring to light any difference between the ploughed and cultivated plots, but on the former, as in the previous year, the plants looked stronger and were a darker green. Numerical estimates of lodging were again made immediately before cutting. Statistical reduction of these figures showed that a significantly ( $P < 0.01$ ) greater proportion of the crop was lodged on the ploughed plots than on either of the other two series; this significant difference can only be attributed to the greater depth of sowing on the ploughed plots giving weaker straw, because the last section of Table II shows that the ploughed plots did not carry any greater weight of straw.

In the final year there was little in appearance to differentiate the three series of plots, except for the thinness of "plant" resulting from the rough surface given by ploughing; there was no lodging.

#### HARVEST DATA

The only significant difference as regards number of ears was that produced in 1932-3 by the poor plant on the ploughed plots; this also was the only experiment where ear size was affected, the ploughed plots showing a significantly higher weight of grain per ear. It should be observed that this latter result cannot be ascribed to the low ear number

(the covariance method allowing for that), and it must be concluded that the conditions on the ploughed plots were definitely conducive to a larger weight of grain per ear.

Turning to the yield of grain (as determined from the whole plots) the results appear contradictory at first sight. In the first and last years there were no significant differences, whilst ploughing gave a higher yield in the second year and the lowest in the third. The results for 1930-1 can probably be attributed to the stronger growth during the winter; that similar conditions in the following year did not produce the same result was undoubtedly due to the greater lodging, which reversed the positions.

The only significant difference in weight of straw was in 1930-1, when the cultivated plots carried 11 per cent. less than the ploughed plots, a difference closely similar to that in grain yield.

#### DISCUSSION

At first sight the end-results of these four experiments do not appear very consistent, but there is no doubt that the apparent contradictions are explicable by the very different conditions at the times when the seed beds were being prepared. In the first year the conditions were very far from normal, and the soil would very rarely be so dry in October; in those extreme circumstances the cultivations used were unimportant and had little effect. The last year was at the other extreme, the October of 1932 being the third wettest in twenty years. This, in conjunction with the fact that the soil texture was more variable than in previous years, led again to conditions that would not frequently occur on potato-growing farms; the only implement which really loosened the ground was the plough, and that left the surface so rough that germination was irregular. Thus in correcting one bad condition another which was equally bad was produced.

In the second and third years the conditions were such as would occur more frequently on ordinary potato-growing farms. The comparison of ploughing and cultivating proceeded similarly in these two years until the summer. In both cases the ploughed plots were appreciably rougher and carried an amount of clod that would be viewed favourably by practical men; this, although it slightly reduced plant establishment and tillering, led to more vigorous growth, and these experiments might be quoted as evidence in support of the belief that an autumn seed bed should not be too fine. In the second year the stronger winter growth was reflected in a definitely higher yield, but in the third it was nullified by

more lodging, the tendency to which is well known to be greater after ploughing than after cultivating.

The following general recommendations, as to the preparation of the seed bed for wheat after potatoes, may be made:

(1) If the land is extremely dry after the potatoes have been removed cultivations will have little effect; the most economic procedure is, therefore, to drill directly.

(2) If the conditions are extremely wet ploughing is desirable, although it may leave the land very rough; on lighter soils it should be possible to correct the condition with some surface working.

(3) In normal conditions the plough should be used because it produces a stronger winter growth. This is subject to the qualification that ploughing favours lodging; it may therefore be better to cultivate if the land is rich and if a weak-strawed variety like Little Joss is grown.

(4) Where a large number of weeds survive the potato-lifting operations it is desirable to plough.

#### SUMMARY

Experiments were carried out in four successive years to compare the plough and the cultivator as implements for preparing a seed bed for wheat after potatoes: in the last two years direct drilling was introduced as a third treatment. Very diverse conditions were experienced, and an attempt has been made to formulate practical recommendations.

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# A NEW METHOD OF ARRANGING VARIETY TRIALS INVOLVING A LARGE NUMBER OF VARIETIES

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(With Two Text-figures)

## I. INTRODUCTION

WHEN a large number of varieties are to be compared in an agricultural field trial the experimenter is confronted with a difficult problem of arrangement. A Latin square containing the whole of the varieties is impossible, being both too unwieldy and requiring too many replications, and even randomised blocks containing the whole of the varieties are unsatisfactory, for the blocks are likely to contain too many plots to eliminate fertility differences efficiently.

A simple method of keeping the block size small is to select one or more varieties as controls and to divide the rest into sets, each set being arranged with the controls in a number of randomised blocks. Unfortunately this method has the disadvantage that comparisons between varieties in different sets are of lower accuracy than comparisons between varieties in the same set. There is the further disadvantage that a disproportionate number of plots is devoted to the control varieties, thus further lowering the efficiency.

The control method can, of course, also be used in conjunction with the Latin square method of design, each set of varieties together with the control or controls being arranged in the form of a Latin square.

Various systematic arrangements of control plots are also possible, the most widely used being that in which the control plot is repeated at fixed intervals along a line of plots. Most frequently in such arrangements a systematic arrangement of the new varieties is also adopted. This is wholly to be condemned, for not only is no valid estimate of error possible, but there is also the disadvantage that differences between neighbouring varieties are likely to be more accurately determined than those between widely separated varieties; moreover, there is a danger of serious disturbance from anything of the nature of a fertility wave. If, however, the new varieties are arranged at random, all these objections are overcome,

so far as the new varieties are concerned; only the comparisons with the controls are now liable to bias and lacking in an estimate of error.

Whatever the exact lay-out of the controls, any method using them suffers from the disadvantage that part of the land has to be devoted to them which would otherwise be devoted to experimental varieties, thus tending to lower the efficiency of the experiment.

To overcome this defect, and at the same time to avoid the use of excessively large blocks, the *pseudo-factorial* type of arrangement, described in this paper, is suggested. In this type of arrangement the varieties are divided into sets for comparison in more than one way, the sets of each division being so arranged that they cut across those of all the other divisions. Thus 100 varieties, numbered 00-99, may be divided into sets of 10 in two ways, the first group of 10 sets consisting of varieties 00-09, 10-19, 20-29, etc., and the second group of 10 sets consisting of varieties 00, 10, 20, ..., 90; 01, 11, 21, ..., 91; 02, 12, 22, ..., 92; etc. Each set of 10 can then be arranged in the field in the form of one or more randomised blocks of 10 plots each, or in the form of a  $10 \times 10$  Latin square, according to the number of replications that are feasible.

Information on the difference of two varieties in the same set, such as varieties 00 and 01, will then accrue from two sources. Firstly there will be the direct comparison within the given set 00-09, and secondly 00 may be compared with the mean of 10, 20, ..., 90, and 01 may be compared with the mean of 11, 21, ..., 91 in the sets of the second group, the difference between the two means being determined from comparisons within the first group of sets. The difference between two varieties such as 00 and 11 not occurring in the same set may also be determined in various ways.

More elaborate arrangements of this type are clearly possible. Instead of two groups of sets three may be employed. With 125 varieties, for instance, three groups of 25 sets of 5 may be used, the first group of 25 sets being made up of varieties 1-5, 6-10, 11-15, ..., 121-125, the second group of varieties 1, 6, 11, 16, 21; 2, 7, 12, 17, 22; ..., 26, 31, 36, 41, 46; ..., and the third group of varieties 1, 26, 51, 76, 101; 2, 27, 52, 77, 102; .... With these three groups of sets, using  $5 \times 5$  Latin squares, 15 replications would be required.

Moreover, the sets of the different groups need not necessarily be of the same size. Thus 90 varieties might be arranged in one group of 9 sets of 10, these being made up of varieties 1-10, 11-20, ..., 81-90, and one group of 10 sets of 9, made up of 1, 11, 21, ..., 81; 2, 12, 22, ..., 82; .... Similar arrangements are possible with three or more groups of sets.

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The object of this paper is to discuss in detail the analysis and efficiency of these different types of pseudo-factorial arrangement. As a preliminary the efficiency of arrangements employing controls will be briefly considered.

### II. CONTROLS IN CONJUNCTION WITH RANDOMISED BLOCKS AND LATIN SQUARES

We will first consider the use of controls in conjunction with ordinary randomised blocks or Latin squares.

Denote the number of plots per block by  $k$ , the number of controls per block by  $c$ , the number of varieties other than controls by  $v$ , the number of replicates of each variety other than controls by  $r$ , and the error variance per plot by  $\sigma^2$ .

The number of sets of varieties is then  $v/(k-c)$ , and the total number of plots  $N$  is  $rkv/(k-c)$ .

The error variances of the various types of comparison are as follows:

Comparison between	Error variance
Two varieties (not controls) in one set	$\frac{2\sigma^2}{r}$
Two varieties not in the same set	$\frac{2\sigma^2}{r} \left(1 + \frac{1}{c}\right)$
Mean of controls and one other variety	$\frac{\sigma^2}{r} \left(1 + \frac{1}{c}\right)$
One control and one other variety	$\frac{\sigma^2}{r} \left(1 + \frac{1}{c}\right) + \frac{\sigma^2}{r} \frac{k-c}{v} \left(1 - \frac{1}{c}\right)$

Since all the comparisons have not the same error variance some criterion for assessing the accuracy of the experiment as a whole will be required. The following suggest themselves:

- (1) The greatest error variance of any comparison.
- (2) The mean error variance of all comparisons.
- (3) The mean of the information on all comparisons, *i.e.* the mean of the reciprocals of the error variances of all comparisons.

Criterion (3), which at first sight appears the logical one, does not, however, take into account the advantages that accrue from having all the comparisons of equal accuracy, and for some purposes (1) might be considered the best single criterion. Criterion (2) has the advantage that it has in general a simpler algebraic expression than (3). It will be somewhat greater than (3), though only very slightly so when the variation in accuracy is only small. In what follows, therefore, we shall limit ourselves to the first two criteria, basing our general conclusions on the second.

To determine the optimum number of controls with a given number of plots per block we shall require to determine the minimum of whatever criterion we consider appropriate. We shall first take as a criterion the greatest error variance of any comparison, namely

$$\frac{2\sigma^2}{r} \left(1 + \frac{1}{c}\right).$$

This is equal to

$$\frac{2\sigma^2 vk (c+1)}{N (k-c) c}.$$

In the case in which the controls are standard varieties we require a value of  $c$  which makes

$$\text{const.} \times \frac{(c+1)}{(k-c) c}$$

a minimum. This is given by

$$c(k-c) + (c+1)(2c-k) = 0,$$

$$c = -1 + \sqrt{1+k}.$$

The mean error variance of all comparisons, including controls, is

$$V_m = \frac{2\sigma^2 k}{N(v+c)(v+c-1)} \left[ \frac{v^2}{c(k-c)} \{(v+c)(c+1) - k\} + (v+c)(c-1) \right].$$

If the controls are themselves new varieties, and if the mean error variance of all comparisons is chosen as the criterion, the equations for  $c$  are more complicated, but will not lead to any widely different values when the number of varieties is large, for comparisons between varieties not in the same set then predominate. In practice only integral values of  $c$  are possible, and after calculating  $c$  from the above equation the relative mean error variances may be calculated for the two nearest integral values of  $c$  in order to decide on which value to adopt.

The efficiency of the arrangement clearly not only depends on the number of controls per block but also on the choice of block size  $k$ , hitherto taken as fixed. The error variance per plot will be some function of the block size, and until this function is known it is not possible to determine the most efficient block size. In general, however, it will be found that so far as arrangements in randomised blocks are concerned the method is unlikely to be much more efficient than randomised blocks including all varieties, and on uniform land may be much less efficient. Since it is always likely to be less efficient than the use of systematic controls and pseudo-factorial arrangements there is no need to investigate the matter in more detail here. It need only be noted that the use of randomly placed controls may make possible the employment of Latin

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squares where these would otherwise be impossible, and for this reason may possess a certain limited usefulness.

In the experiments at Rothamsted and its associated centres, for example, it was found that if no restrictions had been imposed the error variance of the  $5 \times 5$  Latin squares carried out between 1927 and 1934 would have been increased on the average in the ratio 2.49 : 1. If we were conducting an experiment on 25 varieties we might take one as a control and divide the rest into six sets of four, using six  $5 \times 5$  Latin squares in all. We should then have  $V_m = 0.72\sigma^2$ . The corresponding variance for randomised blocks of 25 plots would be  $V_B = \frac{1}{3}\sigma'^2$ . This gives

$$V_B : V_m = 0.462\sigma'^2/\sigma^2 = 1.15.$$

Thus the method of controls is 15 per cent. more efficient than that of randomised blocks.

There is nothing in the pseudo-factorial type of arrangement which prevents the use of Latin squares instead of randomised blocks, but a greater number of replicates will be required, and this may be impracticable. In the above example a  $5 \times 5$  pseudo-factorial arrangement would require ten  $5 \times 5$  Latin squares. Should this number of replicates be possible, however, the pseudo-factorial arrangement (as will be shown later in the paper) would give an efficiency of  $\frac{4}{5} \times 2.49 = 1.66$ , and is thus a much better arrangement than the method of controls.

### III. SYSTEMATIC ARRANGEMENT OF CONTROLS

One of the disadvantages of the use of controls in conjunction with ordinary randomised blocks is that each control plot is used to furnish information only on the fertility of the block in which it occurs, though it may, for example, fall at the edge of that block and be in fact just as good an indicator of the fertility of plots in its neighbourhood belonging to the next block.

Many methods of utilising the information of systematically arranged control plots have been advocated from time to time, some of them very complicated, and most of them open to serious statistical objections. If, however, the control plots are regarded merely as indicators of the fertility in their neighbourhood and a proper randomisation process is applied to the varieties allotted to all the remaining plots, a statistically valid arrangement for the comparison of the varieties other than controls will result, and appropriate methods of analysis are available.

The simplest type of arrangement of this nature is that in which the plots are arranged in one or more lines, the controls being placed at equal

intervals along the lines. Thus, for example, 12 new varieties might be compared in three randomised blocks with controls at every 5th plot. The following arrangement is of this type:

## Block I

$C_1 \quad a \quad l \quad b \quad k \quad C_2 \quad g \quad e \quad f \quad h \quad C_3 \quad c \quad d \quad j \quad i \quad C_4$

## Block II

$C_5 \quad j \quad g \quad l \quad k \quad C_6 \quad c \quad e \quad d \quad b \quad C_7 \quad a \quad f \quad i \quad h \quad C_8$

## Block III

$C_9 \quad k \quad c \quad g \quad a \quad C_{10} \quad i \quad l \quad j \quad b \quad C_{11} \quad f \quad e \quad k \quad d \quad C_{12}$

In analysing such an experiment the experimenter is at liberty to use any function of the controls that he fancies as a measure of the fertility of the neighbouring plots. For practical reasons he will be well advised to choose a simple function, for the advantages accruing from the more complicated functions that have sometimes been suggested are likely to be more theoretical than real.

The simplest function is the mean of the two control plots between which the plot under consideration is situated, but the weighted mean of the two controls, with weights inversely proportional to their distances, would appear to be more suitable. Thus the appropriate values for the first four experimental plots in the above arrangement (varieties  $a, l, b, k$  respectively) would be

$$\frac{1}{3}C_1 + \frac{1}{3}C_2, \quad \frac{2}{3}C_1 + \frac{1}{3}C_2, \quad \frac{1}{3}C_1 + \frac{2}{3}C_2, \quad \frac{1}{3}C_1 + \frac{1}{3}C_2.$$

Having decided on the measure of fertility, the experimenter is equally at liberty to use this measure in whatever way appears desirable. He may take the differences between it and the yields of the experimental plots, or he may express the experimental yields as percentages of the corresponding fertility measures, but here again he will be well advised to adopt some simple procedure; in particular percentages are unlikely to possess any advantages over differences. When the corrected values have been obtained it is only necessary to analyse them in the same manner as the results of an ordinary randomised block experiment.

There is, however, a more effective way of using these fertility measures. Such measures are of the nature of concomitant observations, and can be treated in the same manner as other information of this type, such as the yields of a preliminary uniformity trial, namely, by the procedure of the analysis of covariance. The corrected values will then be of the form  $y_u - bf_u$ , where  $y_u$  is the uncorrected yield of the  $u$ 'th plot,  $f_u$  the

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fertility measure, and  $b$  is a constant whose value is determined from the observations themselves, being such that the error of the corrected yields is minimised. By this procedure the danger of overcorrection for the fertility measures, which will almost certainly occur if straight differences are taken, will be avoided, and only that weight will be attached to the fertility measures which is justified by the results of the particular experiment under consideration.

In cases where the majority of the variation in plot yields is due to causes other than fertility differences, or where the control plots provide an inadequate representation of such differences, a value of  $b$  much smaller than unity may be anticipated, and the covariance method will then be markedly the more accurate. In the limiting case where there is no association between the yields of neighbouring plots, so that the plot errors may be regarded as independent, with, say, a variance  $\sigma^2$ , the variances of the fertility indices of each set of four plots (with controls at intervals of five plots) are  $17/25$ ,  $13/25$ ,  $13/25$  and  $17/25$  times  $\sigma^2$  respectively, the mean being  $\frac{3}{2}\sigma^2$ . The covariance will in this case be zero, and therefore the variance of the differences  $y_u - f_u$  will be  $\frac{8}{5}\sigma^2$ . (With controls at intervals of  $n$  plots this variance equals  $(5n-1)\sigma^2/3n$ .) The method of covariance will give  $b$  zero, and we shall therefore be using the unaltered yields, which will have a variance of  $\sigma^2$ . The loss of efficiency in this case by the use of the differences  $y_u - f_u$  in place of  $y_u - bf_u$  is therefore very considerable, ranging from  $\frac{1}{3}$  when every alternate plot is a control to  $\frac{2}{3}$  when the interval between controls is large.

Apart from the avoidable loss of information due to the use of differences  $y_u - f_u$ , or other arbitrary functions, in place of  $y_u - bf_u$ , allowance must also be made, when comparing the efficiency of systematic controls with ordinary randomised blocks, for the reduction in the number of plots available for the experimental varieties due to the existence of the controls. The ratio of the amounts of information tends in the limiting case just considered to the ratio of the number of non-control plots to the total number of plots.

There is, of course, nothing in the method of controls that precludes the use of a fertility index deduced from controls of more than one line of plots, and in certain cases, particularly with square plots, this may be advantageous. Thus a pattern such as the following might be adopted:

$C_1$	+	+	$C_2$	+	+	$C_3$	+	+	$C_4$
+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
$C_5$	+	+	$C_6$	+	+	$C_7$	+	+	$C_8$

The weighted mean of the four nearest control plots could be taken as the fertility index for plots not in the same row or column as a control, that for the second plot of the second row, for example, being:

$$\frac{1}{3}C_1 + \frac{2}{3}C_2 + \frac{2}{3}C_5 + \frac{1}{3}C_6.$$

Clearly many such patterns are possible. Their relative merits can only be tested by extensive examination of uniformity trials on the type of land and with the type of crop on which it is desired to experiment.

#### IV. PSEUDO-FACTORIAL ARRANGEMENTS IN TWO EQUAL GROUPS OF SETS

For two equal groups of sets to be possible the number of varieties must be a perfect square, say  $p^2$ . The variety in the  $u$ 'th set of the first group and the  $v$ 'th set of the second will be denoted by the pair of numbers  $uv$ . Its mean yield over the replicates of the first group of sets will be denoted by  $x_{uv}$ , and its mean yield over the replicates of the second group of sets by  $y_{uv}$ .

For purposes of computation the mean yields of each group of sets should be set out in squares of the form of Table I.

Table I

First group					Second group						
Set	1	2	...	$p$	Mean	Set	1	2	...	$p$	Mean
	$x_{11}$	$x_{21}$	...	$x_{p1}$	$\bar{x}_{.1}$		$y_{11}$	$y_{21}$	...	$y_{p1}$	$\bar{y}_{.1}$
	$x_{12}$	$x_{22}$	...	$x_{p2}$	$\bar{x}_{.2}$		$y_{12}$	$y_{22}$	...	$y_{p2}$	$\bar{y}_{.2}$
	.....	.....	.....	.....	.....		.....	.....	.....	.....	.....
	$x_{1p}$	$x_{2p}$	...	$x_{pp}$	$\bar{x}_{.p}$		$y_{1p}$	$y_{2p}$	...	$y_{pp}$	$\bar{y}_{.p}$
Mean	$\bar{x}_{1.}$	$\bar{x}_{2.}$	...	$\bar{x}_{p.}$	$\bar{x}_{..}$	Mean	$\bar{y}_{1.}$	$\bar{y}_{2.}$	...	$\bar{y}_{p.}$	$\bar{y}_{..}$

The marginal means, or totals, will be required as indicated. In the actual numerical computations the use of totals is more convenient, but the corresponding algebraic formulae can be more neatly expressed (and remembered) in terms of the means; the substitution of totals in place of means for numerical work will present no difficulty.

The equations for estimating the varietal differences can be derived directly by the classical method of least squares, fitting constants for sets and for varieties. This method will also conveniently provide expressions for the various components of the sum of squares in the analysis of variance. The errors of the various types of comparison are, however, better derived by methods recently developed in connection with the analysis of factorial experiments as described by Fisher<sup>(1)</sup> and Yates<sup>(3)</sup>. We propose, therefore, to use this line of approach in the present paper.



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An experimental arrangement in two equal groups of sets is analogous to a factorial experiment involving two factors (each with  $p$  values) in which the main effects of one factor are confounded in one half (the first group) of the replications and the main effects of the other factor are confounded in the other half (the second group). In such an experiment the  $p^2 - 1$  treatment degrees of freedom are divisible into

First factor	$p - 1$
Second factor	$p - 1$
Interactions	$(p - 1)^2$

The main effects of the first factor will be estimated from the second group of replications only, and those of the second factor from the first group only, the precision of each being one-half that of an unconfounded experiment with the same error variance per plot. The interactions will be unaffected by the confounding and will have estimates of full precision.

In the corresponding pseudo-factorial varietal trial the estimates required are not those of main effects and interactions but differences between single varieties. Such differences must therefore be expressed in terms of the main effects and interactions.

$u \backslash v$	1	2	3	...	$p$
1	$\alpha$	$\beta$			
2	$\gamma$	$\delta$			
3					
...					
$p$					

Fig. 1.

We will first consider the estimate of the deviation of any one variety from the mean of all the varieties. Let the true yields per plot of the varieties be represented by  $\tau_{uv}$ , and take  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  to represent the sums of the  $\tau$ 's contained in the compartments of the two-way table represented in Fig. 1: so that  $\alpha = \tau_{11}$ ,  $\beta = \tau_{21} + \tau_{31} + \dots + \tau_{p1}$ , etc. Further take

$$\begin{aligned}
 A &= (p-1)^2 \alpha - (p-1) (\beta + \gamma) + \delta, \\
 B &= (p-1) (\alpha + \beta) - (\gamma + \delta), \\
 C &= (p-1) (\alpha + \gamma) - (\beta + \delta), \\
 D &= \alpha + \beta + \gamma + \delta.
 \end{aligned}$$

The quantity  $A$  is a component of the interactions of the two factors and is therefore estimated from both groups of replications, *i.e.* from the

mean of the  $x$ 's and  $y$ 's.  $B$  is a component of the main effects of the second factor and is therefore estimated from the first group of replications, *i.e.* from the  $x$ 's only. Similarly  $C$  is estimated from the second group of replications, *i.e.* from the  $y$ 's only.

Now  $p^2\tau_{11} = A + B + C + D$ , and replacing  $D$  by  $p^2\bar{\tau}_{..}$  we have

$$p^2(\tau_{11} - \bar{\tau}_{..}) = A + B + C,$$

and the estimate  $t_{11} - \bar{t}_{..}$  of  $\tau_{11} - \bar{\tau}_{..}$  is therefore given by

$$\begin{aligned} p^2(t_{11} - \bar{t}_{..}) = & (p-1)^2 \frac{1}{2} (\alpha_x + \alpha_y) - (p-1) \frac{1}{2} (\beta_x + \beta_y + \gamma_x + \gamma_y) + \frac{1}{2} (\delta_x + \delta_y) \\ & + (p-1) (\alpha_x + \beta_x) - (\gamma_x + \delta_x) \\ & + (p-1) (\alpha_y + \gamma_y) - (\beta_y + \delta_y), \end{aligned}$$

which after some simplification, replacing  $\bar{t}_{..}$  by  $\frac{1}{2}(\bar{x}_{..} + \bar{y}_{..})$ , reduces to the final generalised expression

$$t_{uv} = \frac{1}{2} (x_{uv} + y_{uv} - \bar{x}_{u.} + \bar{x}_{.v} + \bar{y}_{u.} - \bar{y}_{.v}).$$

Differences between the adjusted values  $t_{uv}$  will give efficient estimates of the varietal differences freed from block effects.

The variance of the difference of two such adjusted means can be determined in a similar manner by considering a  $2 \times 2$  element of four values.

Consider the four values

$$\begin{array}{cc} \tau_{11} & \tau_{21} \\ \tau_{12} & \tau_{22} \end{array}$$

The differences between these may be represented as the main effects and interactions of this two-way table, say

$$\begin{aligned} U &= \frac{1}{2} \{(\tau_{21} + \tau_{22}) - (\tau_{11} + \tau_{12})\}, \\ V &= \frac{1}{2} \{(\tau_{12} + \tau_{22}) - (\tau_{11} + \tau_{21})\}, \\ \{U \cdot V\} &= \frac{1}{2} \{(\tau_{11} + \tau_{22}) - (\tau_{12} + \tau_{21})\}. \end{aligned}$$

Now take  $\alpha'$ ,  $\beta'$ ,  $\gamma'$  and  $\delta'$  to represent the sums of the  $\tau$ 's contained in the compartments of the two-way table represented in Fig. 2:

$v \backslash u$	1	2	3	...	$p$
1	$\alpha'$	$\beta'$			
2					
3					
...					
$p$	$\gamma'$	$\delta'$			

Fig. 2.

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so that  $\alpha' = \tau_{11} + \tau_{12}$ , etc. Further take

$$E = (p-2) (\alpha' - \beta') - 2 (\gamma' - \delta'),$$

$$F = \alpha' - \beta' + \gamma' - \delta'.$$

We have

$$-2pU = p (\alpha' - \beta') = E + 2F.$$

The quantity  $E$  is a component of interaction between the two factors and therefore its estimate has a variance

$$V(E) = \frac{\sigma^2}{r} \{(p-2)^2 4 + 8(p-2)\} = \frac{4\sigma^2}{r} p(p-2),$$

while  $F$  is a component of the main effect of the first factor and therefore its estimate has a variance

$$V(F) = \frac{2\sigma^2}{r} (2p) = \frac{4\sigma^2}{r} p.$$

Hence

$$V(U) = \frac{1}{4p^2} \{V(E) + 4V(F)\} = \frac{\sigma^2}{rp} (p+2) = V(V)$$

and since  $\{U, V\}$  is a component of interaction its estimate has a variance of  $\sigma^2/r$ .

Now

$$\tau_{21} - \tau_{11} = U - \{U, V\}$$

and therefore the variance of the difference of two varieties having a set in common is

$$V(t_{21} - t_{11}) = \frac{\sigma^2}{rp} (p+2) + \frac{\sigma^2}{r} = \frac{2\sigma^2}{r} \cdot \frac{p+1}{p},$$

since all the degrees of freedom concerned are orthogonal.

Similarly the variance of the difference of two varieties not having a set in common is

$$V(t_{22} - t_{11}) = V(U + V) = \frac{2\sigma^2}{r} \cdot \frac{p+2}{p}.$$

It can easily be shown that the mean variance of all comparisons is equal to twice the expectation of the sum of squares of deviations between all adjusted varietal means divided by the associated number of degrees of freedom,  $p^2 - 1$ , thus providing a check. Resolving this sum of squares into its component parts we have the following expectations:

	Degrees of freedom	Expectation of mean square	Expectation of sum of squares
First factor	$p-1$	$2\sigma^2/r$	$2\sigma^2(p-1)/r$
Second factor	$p-1$	$2\sigma^2/r$	$2\sigma^2(p-1)/r$
Interactions	$(p-1)^2$	$\sigma^2/r$	$\sigma^2(p-1)^2/r$
Total	$p^2-1$		$\sigma^2(p+3)(p-1)/r$

Thus the mean variance of all comparisons is

$$V_m = \frac{2\sigma^2}{r} \cdot \frac{p+3}{p+1}.$$

Had there been no confounding the variance of every comparison would have been  $2\sigma^2/r$ . The factor  $(p+3)/(p+1)$  is therefore a measure of the increase in variance that results from the division of the varieties into sets when the error variance per plot is unaltered by the resultant reduction in block size. Its reciprocal, which may be called the *efficiency factor* of the arrangement, is a measure of the inherent strength of the arrangement. Values of this factor for various numbers of varieties are given in Table II, which also includes values of the similar factor for two groups of unequal sets described in section VII, and for the Latin square type of arrangement described in section VI, this latter being

$$(p+1)/(p+2\frac{1}{2}).$$

Table II. *Efficiency factors in two-dimensional arrangements*

No. of varieties Arrangement	25 5 <sup>2</sup>	30 5 × 6	36 6 <sup>2</sup>	42 6 × 7	49 7 <sup>2</sup>	64 8 <sup>2</sup>	100 10 <sup>2</sup>	144 12 <sup>2</sup>	256 16 <sup>2</sup>	400 20 <sup>2</sup>
Two groups of sets	0.75	0.764	0.778	0.788	0.8	0.818	0.846	0.867	0.895	0.913
Latin square grouping	0.8	—	0.824	—	0.842	0.857	0.880	0.897	0.919	0.933
Random controls	0.485	—	0.511	—	0.532	0.547	0.568	0.596	0.631	0.658

The table also shows the similar efficiency factors for the method of random controls described in section II. Since the error variance per plot is the same for all three types of arrangement the comparison of the three factors gives the relative efficiency of the three methods. It will be seen that the method of random controls is always very much less efficient than the corresponding pseudo-factorial arrangements.

The partition of the degrees of freedom in the analysis of variance is shown in Table III for the case of randomised blocks.

Table III

		Degrees of freedom
Blocks	Between groups	1
	Between sets	$p-1$
	Within sets	$p-1$
		$p(\frac{1}{2}r-1)$
Varieties	First factor	$p-1$
	Second factor	$p-1$
	Interactions	$(p-1)^2$
		$(p-1)^2$
Error	Between groups	$p(p-1)(\frac{1}{2}r-1)$
	Within group I	$p(p-1)(\frac{1}{2}r-1)$
	Within group II	$p(p-1)(\frac{1}{2}r-1)$
		$p^2r-1$
Total		$p^2r-1$

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The first factor component of the varietal sum of squares is computed from the values of group II only, and the second factor component from group I only. The varietal sums of squares are therefore equal to

$$\text{First factor: } \frac{1}{2}r (pS\bar{y}_{u..}^2 - p^2S\bar{y}_{..}^2).$$

$$\text{Second factor: } \frac{1}{2}r (pS\bar{x}_{.v}^2 - p^2S\bar{x}_{..}^2).$$

$$\text{Interactions: } \frac{1}{4}r \{SS(x+y)^2 - pS(\bar{x}_{u.} + \bar{y}_{u.})^2 - pS(\bar{x}_{.v} + \bar{y}_{.v})^2 + p^2(\bar{x}_{..} + \bar{y}_{..})^2\}.$$

The sum of squares for sets and groups is

$$\frac{1}{2}r \{p(S\bar{x}_{u.}^2 + S\bar{y}_{.v}^2) - \frac{1}{2}p^2(\bar{x}_{..} + \bar{y}_{..})^2\}.$$

If these four sums of squares are added together they total

$$\frac{1}{4}r \{SS(x+y)^2 + pS(\bar{x}_{u.} - \bar{y}_{u.})^2 + pS(\bar{x}_{.v} - \bar{y}_{.v})^2 - p^2(\bar{x}_{..} - \bar{y}_{..})^2 - p^2(\bar{x}_{..} + \bar{y}_{..})^2\}$$

which is the best form for computation, the sum of squares for varieties being obtained by subtraction of the sum of squares for sets and groups. The sum of squares for error between groups can likewise be obtained by subtraction of this total from the total sum of squares between all  $x$  and  $y$ , namely

$$\frac{1}{2}r \{SSx^2 + SSy^2 - p^2(\bar{x}_{..} + \bar{y}_{..})^2\}.$$

#### V. THREE-DIMENSIONAL PSEUDO-FACTORIAL ARRANGEMENTS IN THREE EQUAL GROUPS OF SETS

The number of varieties must be a perfect cube, say  $p^3$ . We shall only consider the type of arrangement in which each variety is specified by three co-ordinate numbers,  $u, v, w$ ,  $uv$  denoting the set out of the  $p^2$  of the first group in which the variety is located,  $uw$  and  $vw$  the similar sets of the second and third groups respectively. The mean yields of the variety in the  $\frac{1}{3}r$  replicates of each of the three groups will be denoted by  $x_{uvw}, y_{uvw}, z_{uvw}$  respectively.

Each of these three groups of yields may be arranged in the form of a cube. The marginal means of each of these will be required and can be denoted by an obvious extension of the previous notation. The cube formed from the means,  $m_{uvw}$ , of the corresponding values of these three cubes will also be required.

By a similar process to that of the last section it will be found that the adjusted varietal means are given by

$$t_{uvw} = m_{uvw} + \frac{1}{2}(\bar{m}_{.vw} + \bar{m}_{u.w} + \bar{m}_{uv.}) - \frac{1}{2}(\bar{m}_{u..} + \bar{m}_{.v.} + \bar{m}_{..w}) \\ - \frac{1}{2}(\bar{x}_{.vw} + \bar{y}_{u.w} + \bar{z}_{uv.}) + \frac{1}{2}(\bar{x}_{u..} + \bar{y}_{.v.} + \bar{z}_{..w}),$$

which for computation is best written in the form

$$t_{uvw} = m_{uvw} + c_{.uw} + c_{u.w} + c_{uv.},$$

where  $c_{.uw} = \frac{1}{2}(\bar{m}_{.vw} - \bar{x}_{.vw} - \bar{m}_{.v.} + \bar{y}_{.v.})$ , etc.

Following the same procedure as in the previous section we may represent the differences of the cube of the eight  $\tau$ 's,  $\tau_{111} \dots \tau_{222}$ , by the main effects  $U$ ,  $V$ ,  $W$ , and the interactions  $\{U.V\}$ ,  $\{U.W\}$ ,  $\{V.W\}$ ,  $\{U.V.W\}$  of the three-way table, each of these quantities being  $\frac{1}{4}$  of the sum of four of the  $\tau$ 's less the sum of the other four.

It will be found that

$$\begin{aligned} V(U) &= V(V) = V(W) = \frac{\sigma^2}{2rp^2} (p^2 + 2p + 4), \\ V(\{U.V\}) &= V(\{U.W\}) = V(\{V.W\}) = \frac{\sigma^2}{2rp} (p + 1), \\ V(\{U.V.W\}) &= \frac{\sigma^2}{2r}. \end{aligned}$$

Since

$$\begin{aligned} \tau_{211} - \tau_{111} &= U - \{U.V\} - \{U.W\} + \{U.V.W\}, \\ \tau_{122} - \tau_{111} &= V + W - \{U.V\} - \{U.W\}, \\ \tau_{222} - \tau_{111} &= U + V + W + \{U.V.W\}, \end{aligned}$$

we obtain

$$\begin{aligned} V(t_{211} - t_{111}) &= \frac{2\sigma^2}{rp^2} (p^2 + p + 1), \\ V(t_{122} - t_{111}) &= \frac{\sigma^2}{rp^2} (2p^2 + 3p + 4), \\ V(t_{222} - t_{111}) &= \frac{\sigma^2}{rp^2} (2p^2 + 3p + 6). \end{aligned}$$

The mean variance of all comparisons is found to be

$$V_m = \frac{\sigma^2}{r} \frac{2p^2 + 5p + 11}{p^2 + p + 1}.$$

The efficiency of this type of arrangement is therefore

$$2(p^2 + p + 1)/(2p^2 + 5p + 11).$$

The values of this factor for various numbers of varieties (including some involving unequal sets, as described in section VIII) are shown in Table IV, which also gives the corresponding factors for the method of controls for comparison.

Table IV. *Efficiency factors in three-dimensional arrangements*

No. of varieties Arrangement	64 4 <sup>3</sup>	80 4 <sup>2</sup> × 5	100 4 × 5 <sup>2</sup>	125 5 <sup>3</sup>	216 6 <sup>3</sup>	343 7 <sup>3</sup>	512 8 <sup>3</sup>
Factorial arrangement	0.667	0.684	0.702	0.721	0.761	0.792	0.816
Random controls	0.393	—	—	0.416	0.455	0.484	0.506

The efficiency factors of these three-dimensional arrangements are decidedly lower than those of the two-dimensional arrangements given

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in Table II. This, and the greater computational labour they entail, must be offset against the greater reduction in block size.

The partition of the degrees of freedom is similar to that already given for the classification into two groups of sets. The sum of squares due to treatments in the analysis of variance can be built up from its components in the same manner as before, but for practical purposes it is best calculated from the formula

$$rSSSt_{uvw}m_{uvw} - \frac{1}{3}rpSS(\bar{t}_{.vw}\bar{x}_{.vw} + \bar{t}_{u.w}\bar{y}_{u.w} + \bar{t}_{uv.}\bar{z}_{uv.}).$$

The sum of squares due to the component of error between groups can be calculated by deducting this, and the sums of squares due to sets, groups and mean, which three latter together total

$$\frac{1}{3}rpSS(\bar{x}_{.vw}^2 + \bar{y}_{u.w}^2 + \bar{z}_{uv.}^2),$$

from the total sum of squares arising from the values of the three cubes, namely,

$$\frac{1}{3}rSSS(x_{uvw}^2 + y_{uvw}^2 + z_{uvw}^2).$$

### VI. TWO-DIMENSIONAL PSEUDO-FACTORIAL ARRANGEMENTS IN THREE GROUPS OF SETS FORMING A LATIN SQUARE

If a number of varieties forming a perfect square is classified in two ways as in Table I, and in addition in a third way such that every set of the third group contains one and only one variety from each of the sets of the first group and also from each of the sets of the second group, so that the three classifications fulfil the conditions of a Latin square, each set of varieties in each of the three groupings may be compared by means of randomised blocks or a Latin square.

The analytical solution remains comparatively simple. Denote each of the  $p^2$  varieties by three numbers  $u, v, w$  (indicating the sets of the three groups to which that variety belongs) and the mean yields for the three groupings by  $x_{uvw}, y_{uvw}$  and  $z_{uvw}$  respectively, their mean being  $m_{uvw}$ . It will be noted that any two of the three numbers are sufficient to specify the variety.

The estimates of the varietal differences are given by differences of the quantities

$$t_{uvw} = m_{uvw} + \frac{1}{2}(\bar{m}_{u..} + \bar{m}_{.v.} + \bar{m}_{..w}) - \frac{1}{2}(\bar{x}_{u..} + \bar{y}_{.v.} + \bar{z}_{..w}).$$

The variance of the differences of two varieties occurring in the same set of one of the three groupings is given by

$$V(t_{uvw} - t_{u'v'w'}) = \frac{2\sigma^2}{r} \left(1 + \frac{1}{p}\right),$$

as in the classification into two groups of sets, and the variance of the difference of two varieties not occurring in the same set is given by

$$V(t_{uvw} - t_{u'v'w'}) = \frac{2\sigma^2}{r} \left(1 + \frac{3}{2p}\right).$$

The mean variance of all comparisons is

$$V_m = \frac{2\sigma^2}{r} \cdot \frac{p + 2\frac{1}{2}}{p + 1}.$$

The sum of squares for (mean + groups + sets + treatments) is equal to

$$r \left[ SS m_{uvw}^2 + \frac{1}{2}p \{S(\bar{x}_{u..} - \bar{m}_{u..})^2 + S(\bar{y}_{.v.} - \bar{m}_{.v.})^2 + S(\bar{z}_{..w} - \bar{m}_{..w})^2\} \right. \\ \left. - \frac{p^2}{6} \{(\bar{x}... - \bar{m}...)^2 + (\bar{y}... - \bar{m}...)^2 + (\bar{z}... - \bar{m}...)^2\} \right].$$

This can be divided into its components in the same manner as the classifications into two groups of sets.

It will be noted that the variance of differences between treatments not in the same set is reduced by the comparisons of the third classification, but that the variance of differences between treatments in the same set remains the same, having as before the factor  $\left(1 + \frac{1}{p}\right)$ . Now when the number of varieties is the square of a prime number (and also in certain other cases) further divisions in sets can be made such that each set contains one and only one variety of every set in every other group. (Each group of sets will constitute with each of the other groups a Graeco-Latin square.) If all the  $p + 1$  such groups of sets are included in the arrangement, every variety occurs with every other variety in one set and one only. Consequently all comparisons are of equal accuracy, and have in fact a variance of  $\frac{2\sigma^2}{r} \left(1 + \frac{1}{p}\right)$  equal to that of the most accurate comparison in the divisions into two and three groups of sets(4).

## VII. PSEUDO-FACTORIAL ARRANGEMENTS IN TWO UNEQUAL GROUPS OF SETS

So far we have considered only arrangements in which the number of varieties is the same in all sets. This necessitates that the number of varieties included in the experiment is a perfect square or a perfect cube. This limitation may be overcome if the number in each set is different for the different groups. In particular a two-factor classification may have  $q$  varieties in each of the  $p$  sets of the first group, and  $p$  varieties in



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each of the  $q$  sets of the second group, giving  $pq$  varieties in all, with  $u$  ranging from 1 to  $p$  and  $v$  from 1 to  $q$ .

An additional difficulty immediately presents itself in arrangements of this type. For since the sets in the two groups are of unequal size the blocks or Latin squares by which the comparisons within sets are made will also be of unequal size, and consequently the error variances per plot ( $\sigma_x^2$  and  $\sigma_y^2$  say) cannot be assumed equal for both groups.

Take the weights of the varietal means  $x_{uv}$  and  $y_{uv}$  of the two groups to be  $\lambda$  and  $\mu$  respectively, so that  $\lambda = r/2\sigma_x^2$  and  $\mu = r/2\sigma_y^2$ . The quantities  $A$ ,  $B$  and  $C$  of section IV must be replaced by

$$\begin{aligned} A &= (p-1)(q-1)\alpha - (q-1)\beta - (p-1)\gamma + \delta, \\ B &= (q-1)(\alpha + \beta) - (\gamma + \delta), \\ C &= (p-1)(\alpha + \gamma) - (\beta + \delta). \end{aligned}$$

In estimates involving both  $x$  and  $y$  the weighted mean must be taken. Thus the quantity  $\frac{1}{2}(\alpha_x + \alpha_y)$  must be replaced by  $(\lambda\alpha_x + \mu\alpha_y)/(\lambda + \mu)$ , etc. Estimates involving only  $x$  or  $y$  will remain unchanged.

This leads by the same procedure as before to the expression

$$(\lambda + \mu)t_{uv} = \lambda x_{uv} + \mu y_{uv} - \lambda(\bar{x}_u - \bar{y}_u) + \mu(\bar{x}_v - \bar{y}_v).$$

In order to obtain the variance of differences of the  $t$ 's  $E$  must be redefined as

$$E = (q-2)(\alpha' - \beta') - 2(\gamma' - \delta'),$$

giving  $-2qU = E + 2F$ , and

$$V(E) = \frac{4q(q-2)}{\lambda + \mu}, \quad V(F) = \frac{2q}{\mu};$$

so that

$$V(U) = \frac{1}{q} \left[ \frac{2}{\mu} + \frac{q-2}{\lambda + \mu} \right],$$

$$V(V) = \frac{1}{p} \left[ \frac{2}{\lambda} + \frac{p-2}{\lambda + \mu} \right],$$

$$V\{U, V\} = \frac{1}{\lambda + \mu}.$$

Thus

$$V(t_{21} - t_{11}) = \frac{2}{q} \left[ \frac{1}{\mu} + \frac{q-1}{\lambda + \mu} \right],$$

$$V(t_{12} - t_{11}) = \frac{2}{p} \left[ \frac{1}{\lambda} + \frac{p-1}{\lambda + \mu} \right],$$

$$V(t_{22} - t_{11}) = \frac{2}{pq} \left[ \frac{q}{\lambda} + \frac{p}{\mu} + \frac{pq - p - q}{\lambda + \mu} \right],$$

the mean variance of all comparisons being

$$V_m = \frac{2}{pq-1} \left[ \frac{q-1}{\lambda} + \frac{p-1}{\mu} + \frac{(p-1)(q-1)}{\lambda + \mu} \right].$$

If the difference in weight is ignored and the  $t$ 's estimated by the unweighted expression of section IV all components estimated from both  $x$  and  $y$  will be estimated from the unweighted means and will therefore have their variances increased in the ratio

$$\frac{1}{4} \left( \frac{1}{\lambda} + \frac{1}{\mu} \right) : \frac{1}{\lambda + \mu} = \frac{(\lambda + \mu)^2}{4\lambda\mu}.$$

Consequently the variances of the differences of the unweighted  $t$ 's may be immediately obtained by replacing the fraction  $1/(\lambda + \mu)$  by  $(\lambda + \mu)/4\lambda\mu$  wherever it occurs. The fractional increase of these components will therefore be

$$\frac{(\lambda + \mu)^2}{4\lambda\mu}$$

and the fractional increases in the total variances will therefore always be slightly less than this.

The percentage values of this fraction are

$\lambda : \mu$	2 : 1	3 : 2	4 : 3	5 : 4	6 : 5
Percentage	12.5	4.2	2.1	1.2	0.83

The loss of information by the use of unweighted means is therefore very small provided the error variances of the two groups are not widely different. This is usually the case in practice if  $p$  and  $q$  are not widely different. Nor is there ever any reason why they should be. With 90 varieties, for instance, a  $9 \times 10$  arrangement would be chosen in preference to a  $6 \times 15$  arrangement, since the nearer the arrangement approaches to symmetry the more efficient it will be. With 95 varieties a  $10 \times 10$  arrangement with 5 additional varieties would undoubtedly be better than a  $19 \times 5$  arrangement and probably better than a  $12 \times 8$  arrangement with one additional variety.

When the weights are equal, so that  $\lambda = \mu = r/2\sigma^2$ , the variances of the varietal comparisons become

$$\begin{aligned} V(t_{21} - t_{11}) &= \frac{2\sigma^2}{r} \left( 1 + \frac{1}{q} \right), \\ V(t_{12} - t_{11}) &= \frac{2\sigma^2}{r} \left( 1 + \frac{1}{p} \right), \\ V(t_{22} - t_{11}) &= \frac{2\sigma^2}{r} \left( 1 + \frac{1}{p} + \frac{1}{q} \right), \\ V_m &= \frac{2\sigma^2}{r} \frac{(p+1)(q+1) - 4}{pq - 1}. \end{aligned}$$

If the weights are unequal these expressions may be used without appreciable error in place of the exact expressions in all cases in which it is admissible to ignore the differences of weights.

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There is another cause of unequal error variance in the two groups. If these occupy different parts of the field it may be that the fertility differences of one part are less adequately eliminated by the blocks than those of the other part, or one part of the field may be more irregular than the other part. Consequently it is advisable to randomise the sets of treatments allotted to the different blocks (in so far as this is compatible with the arrangement of the blocks) whether  $p$  and  $q$  are unequal or not. This has been done in the examples which follow.

Finally, if the comparisons within sets are made by means of Latin squares instead of randomised blocks, the number of replicates in the two groups will be unequal, being  $p$  and  $q$  respectively, giving rise to a further inequality in weights, though this inequality is likely to be compensated to a certain extent by a lower error variance in the smaller squares. Even in this case, therefore, weighting will not always be necessary, though it would seem advisable to examine the two components of error within sets before making a decision.

### VIII. THREE-DIMENSIONAL PSEUDO-FACTORIAL ARRANGEMENTS IN THREE UNEQUAL GROUPS OF SETS

Let the varieties be classified according to three numbers  $u, v, w$ , ranging from 1 to  $p, 1$  to  $q$  and 1 to  $r$  respectively, the corresponding weights being  $\lambda, \mu$  and  $\nu$ .

Arguments similar to those developed in the last section apply here also. The exact expressions for  $t_{uvw}$  are given by

$$(\lambda + \mu + \nu) t_{uvw} = \lambda (x_{uvw} - \bar{x}_{.vw}) + \frac{\lambda\nu}{\lambda + \mu} (\bar{x}_{uv.} - \bar{x}_{.v.}) + \frac{\lambda\mu}{\lambda + \nu} (\bar{x}_{u.w} - \bar{x}_{.w}) \\ + \bar{x}_{u..} \left( \frac{\mu\nu}{\lambda + \mu} + \frac{\mu\nu}{\lambda + \nu} \right) + \text{similar terms in } y \text{ and } z.$$

The variances of the different types of comparison are

$$V(t_{211} - t_{111}) = \frac{2}{qr} \left[ \frac{1}{\lambda} + \frac{q-1}{\lambda + \mu} + \frac{r-1}{\lambda + \nu} + \frac{(q-1)(r-1)}{\lambda + \mu + \nu} \right], \\ V(t_{122} - t_{111}) = \frac{2}{pqr} \left[ \frac{q}{\mu} + \frac{r}{\nu} + \frac{q(p-1)}{\lambda + \mu} + \frac{r(p-1)}{\lambda + \nu} \right. \\ \left. + \frac{qr - q - r}{\mu + \nu} + \frac{(qr - q - r)(p-1)}{\lambda + \mu + \nu} \right], \\ V(t_{222} - t_{111}) = \frac{2}{pqr} \left[ \frac{p}{\lambda} + \frac{q}{\mu} + \frac{r}{\nu} + \frac{qr - q - r}{\mu + \nu} + \frac{pr - p - r}{\lambda + \nu} \right. \\ \left. + \frac{pq - p - q}{\lambda + \mu} + \frac{(p-1)(q-1)(r-1) + 1}{\lambda + \mu + \nu} \right],$$

with similar expressions for  $V(t_{121} - t_{111})$ , etc.

If the differences in weights are ignored the fractional increase in components with divisor  $\lambda + \mu$  will be  $(\lambda - \mu)^2/4\lambda\mu$  as before, with similar increases with the divisors  $\lambda + \nu$  and  $\mu + \nu$ . The fractional increase in the components with the divisor  $\lambda + \mu + \nu$  will be

$$\frac{1}{9} \left( \frac{1}{\lambda} + \frac{1}{\mu} + \frac{1}{\nu} \right) (\lambda + \mu + \nu) - 1 = \frac{(\mu - \nu)^2}{9\mu\nu} + \frac{(\lambda - \nu)^2}{9\lambda\nu} + \frac{(\lambda - \mu)^2}{9\lambda\mu}.$$

The loss of information due to the use of unweighted means will therefore be of the same order as in the case of two unequal groups of sets. In practice  $p$  need not differ by more than one unit from  $q$  and  $r$ , which can be equal. This makes available a useful set of values for the total number of varieties which is not effectively increased by the inclusion of a greatest difference of 2 units between  $p$ ,  $q$  and  $r$ . The possible numbers between  $4^3$  and  $8^3$  are:

64, 80, 100, 125, 150, 180, 216, 252, 294, 343, 392, 448, 512.

When the weights are equal the variances of varietal comparisons are:

$$V(t_{211} - t_{111}) = \frac{\sigma^2/r'}{qr} (2qr + q + r + 2),$$

$$V(t_{122} - t_{111}) = \frac{\sigma^2/r'}{pqr} (2pqr + pq + pr + qr + 2q + 2r),$$

$$V(t_{222} - t_{111}) = \frac{\sigma^2/r'}{pqr} (2pqr + pq + pr + qr + 2p + 2q + 2r).$$

$$V_m = \frac{\sigma^2/r'}{pqr - 1} \{ 2(p-1)(q-1)(r-1) + 3(q-1)(r-1) \\ + 3(p-1)(r-1) + 3(p-1)(q-1) \\ + 6(p-1) + 6(q-1) + 6(r-1) \},$$

$r'$  being the number of replications.

## IX. NUMERICAL EXAMPLES

In order to illustrate the various types of arrangement that are discussed in this paper we will take as an example the preliminary results of an experimental orchard of orange trees at the University of California Citrus Experiment Station at Riverside. The orchard was laid out for fertiliser trials, but during the first ten years of its existence was run as a uniformity trial without treatment.

The trial is fully reported and the results discussed by Parker and Batchelor (2). There were 199 plots in all, each of which contained eight trees in a single row, separated by a row of non-experimental oranges and grape fruit from the neighbouring plots. Each tree occupied a square of

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area 0.011 acre. Every attempt was made to select uniform land and to eliminate variability due to rootstocks and scions.

Table V. *Mean annual yields in pounds per tree*

	M	L	K	J	I	H	G	F	E	D
2	87*	103*	96*	103*	113*				100*	121*
4	95	106	119	104	120				127	140
6	111	110	109	99	140				116	141
8	79	112	99	115	129				139	137
10	98	113	116	110	122				130	128
12	102*	109*	122*	128*	113*				140	133*
14	93	126	137	128	108	132*			133*	138
16	100	116	129	119	113	130			100	107
18	93	115	112	119	120	132			124	105
20	98	116	127	135	125	131			116	105
22	98*	138*	132*	137	138*	116	120*		122	124*
24	103	†	121	119*	115	114*	123		118*	121
26	97	121	104	110	117	115	103			130
28	119	119	129	132	129	138	141			116
30	108	131	148	130	152	153	140			136
32	127	127*	135	134	126	129	133*	152*		139
34	119*	†	140*	134*	121*	139*	135	128		128*
36	135	147	146	147	150	143	127	138		136
38	120	137	119	124	136	142	148	129		133
40	121	138	135	139	142	150	133	131		122
42	114	128	157	146	144	142*	155	153*		138
44	107*	126*	143*	135*	126*	†	144*	165		144*
46						126	155	155		146
48						129	140	140		117
50						134	142	131		115
52						114	150	149*		122
54						114*	136*	†		108*

\* Used as controls.

† Yield missing.

Table V shows the mean yields of each plot from 1922 to 1927. The arrangement of the values in the table corresponds to the arrangement of the plots on the ground. The values of four of the plots were discarded for various reasons.

Table VI. *Residual mean squares for blocks of various sizes*

No. of plots per block	No. of blocks	Residual mean square	Plots discarded
199	1	247.43	—
64	3	219.15	L 24†, L 34†, H 44†, F 54†, D 2, D 4, D 6
49	4	192.16	F 54†, E 22, E 24
8	24	119.51	As for blocks of 64
7	28	121.65	As for blocks of 49
4	48	100.89	As for blocks of 64
2	96	104.70	As for blocks of 64

† Yield missing.

The residual mean squares after eliminating block differences are shown for blocks of various sizes in Table VI. These particular block sizes are those required for the examples we shall consider.

The residual mean square is reduced consistently by decrease in block size, except for blocks of two plots and of seven plots. About  $2\frac{1}{2}$  times as much information is available on comparisons within blocks of four plots as is available on the average of all comparisons.

The mean square for blocks of two plots is not significantly larger than the mean square for blocks of four plots, but the reversal of the tendency to decrease is interesting, and may possibly indicate the existence of competition effects.

The following arrangements will be considered in detail:

- (a) The comparison of 49 varieties using systematic controls.
- (b) The comparison of 49 varieties in a  $7 \times 7$  pseudo-factorial arrangement.
- (c) The comparison of 64 varieties in a  $4 \times 4 \times 4$  pseudo-factorial arrangement.

The relative accuracy of these and other possible arrangements will also be discussed.

#### (a) *Systematic controls*

In order to illustrate the method of systematic controls these have been taken at intervals of five or six plots. The actual plots selected are marked with an asterisk in Table V. The choice was made without reference to the yields, and where necessary by random selection. Missing plots were omitted, though it may be noted that by ill chance all four missing plots would have been chosen as controls.

The weighted means of the two neighbouring control plots may now be calculated for each of the experimental plots. Those of M 4-10, for instance, are 90, 93, 96, 99, and those of H 44-52 are [137.33], 132.67, 128, 123.33, 118.67, that in square brackets corresponding to the missing plot H 44. These means will be taken as the fertility indices of the plots. Fractions may be avoided by the use of multiples in the actual analyses.

The analyses of variance and covariance of the fertility indices and the corresponding actual yields are shown in Table VII, which also shows the analyses of variance for the differences of the indices and the yields and for the quantities  $y - bf$ . The value of  $b$  is given by

$$14817.69/19885.13 = 0.7452.$$

The residual mean square for the differences equals 149.78, and that for

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the quantities  $y - bf$  equals 141·90, the degrees of freedom being reduced by 1 in the second case.

In this example the value of  $b$  is not very far removed from unity; the difference between the two errors is consequently not very large, and the covariance method gives only 5 per cent. more information.

Table VII. *Analyses of variance and covariance of fertility indices ( $f$ ) and plot yields ( $y$ )*

		Sums of squares and products				
		$f^2$	$fy$	$y^2$	$(y - f)^2$	$(y - bf)^2$
Blocks	2	5271·21	4768·10	4773·04	508·05	593·95
Residual	146	19885·13	14817·69	31617·63	21867·38	20576·02
Total	148	25156·34	19585·79	36390·67	22375·43	21169·97

To make allowance for the loss of information due to the reduction in the number of available plots these error variances must be multiplied by 195/149 (*i.e.* total number of plots/number of non-control plots) before comparison with that obtained in ordinary randomised blocks of 49 plots. The covariance method thus gives a value of 185·70 as compared with 192·16 for randomised blocks of 49 plots.

### (b) $7 \times 7$ pseudo-factorial arrangement

The plots were divided without reference to the yields into 28 blocks of seven plots each. The particular set of varieties assigned to each block was chosen at random from all the sets for the reasons indicated at the end of section VII. The varieties within each set were then assigned to the plots of the chosen block at random.

Table VIII indicates the resulting arrangement, and should be read in conjunction with Table V. Three of the plots with missing yields have been included in the arrangement, as would be the case in practice.

When some of the yields are missing the correct way of conducting the analysis is to represent these missing yields by symbols, form the error mean square in the ordinary way, and determine the values of the missing yields which make this error mean square a minimum. The analysis can then be completed using these values.

There is no great difficulty in this procedure, but a simpler one is available which will usually give satisfactory results. This consists of giving a value to each missing yield which minimises the part of the error mean square contributed by all the replicates of the set for which the yield is missing. In the example before us there are only two replicates of each set, and this will therefore be effected when the difference between

the value of the missing yield and that of the other replicate of the same variety is equal to the mean difference between the two replicates of all other varieties of these two sets.

Table VIII. *Arrangement of blocks and varieties: 7 × 7 pseudo-factorial arrangement*

	M	L	K	J	I	H	G	F	E	D
2	77	75	74	73	72				71	76
4	52	73	72	57	44				73	43
6	72	71	42	51	43				63	33
8	32	77	12	55	47				56	23
10	62	75	22	53	45				46	13
12	12	72	62	52	42				26	53
14	22	74	32	54	41	26			16	63
16	42	76	52	56	46	16			76	67
18	23	23	75	71	36	46			36	61
20	13	21	25	31	34	56			66	65
22	73	26	45	61	32	66	55		*	66
24	53	22†	65	11	37	76	51		*	64
26	43	24	15	21	33	36	56			62
28	33	27	35	41	35	11	52			34
30	63	25	55	51	31	17	57			54
32	26	45	14	77	57	15	54	17		64
34	22	46†	64	37	37	13	53	13		74
36	27	42	74	47	47	14	34	14		44
38	21	47	24	67	27	12	32	12		14
40	25	43	34	27	67	16	36	11		24
42	24	44	44	57	17	51	31	16*		55
44	23	41	54	17	77	71†	35	15		65
46						61	33	17		75
48						31	37	11		15
50						21	66	13		45
52						41	65	12		35
54						11	64	*†		25

\* Discarded.

† Yield missing.

Blocks of 49 plots: (1) M, L, K 2-10; (2) K 12-44, J, I 2-20; (3) I 22-44, H, G 22-52; (4) G 54, F 32-52, E, D.

Here the yield of plot 24 of column L is missing. This plot corresponds to variety 22 in the first grouping, and the mean of all the other yields in this block is 123.3. The other replicate of 22 in the first grouping is the plot 34 of column M with yield 119, the mean of all the other plots in the block being 120.7. The difference is  $123.3 - 120.7 = 2.6$  and the required yield is therefore  $119 + 2.6 = 122$  say. The values of plot 34 of column L and plot 44 of column H are similarly found to be 125 and 118 respectively.

This procedure will slightly inflate the mean squares for treatments



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and for error between groups, their average value (when treatments produce no effect) and the average value of the mean square for error within groups being approximately in the ratio of 99 : 96. It will, moreover, sacrifice some of the available information on the varieties with missing plots. In this respect it will be roughly equivalent to the process of estimating a quantity  $\zeta$  from three measures  $z_1$ ,  $z_2$  and  $z_3$  of equal

Table IX.  $7 \times 7$  arrangement: sums of yields (less 100) of pairs of replicates in same grouping

First group ( $2y_{uv}$ )								
Set	1	2	3	4	5	6	7	Total
	69	36	107	34	22	45	10	323
	71	41*	86	60	69	79	22	428
	...	...	...	...	...	...	...	...
Total	590	305	498	373	298	433	115	2612
Second group ( $2y_{ur}$ )								
Set	1	2	3	4	5	6	7	Total
1	33	44	64	46	72	63	37*	359
2	1	9	16	9	24	20	30	109
	...	...	...	...	...	...	...	...
Total	291	306	336	391	439	349	298	2410
* Including estimate of missing value.								
Sum of both groups								
$\begin{smallmatrix} u \\ v \end{smallmatrix}$	1	2	3	4	5	6	7	Total $\frac{1}{2}(\bar{x}_{.v} - \bar{y}_{.v})$
1	102	80	171	80	94	108	47	682 - 1.3
2	72	50	102	69	93	99	52	537 + 11.4
	...	...	...	...	...	...	...	...
Total	881	611	834	764	737	782	413	5022 + 7.2
$\frac{1}{2}(\bar{y}_{u.} - \bar{x}_{u.}) - 10.7$	0.0		- 5.8	+ 0.6	+ 5.0	- 3.0	+ 6.5	- 7.2
Adjusted values of treatment means ( $t_{uv}$ )								
$\begin{smallmatrix} u \\ v \end{smallmatrix}$	1	2	3	4	5	6	7	
1	113.5	118.7	135.6		119.3	127.2	122.7	117.0
2	118.7	123.9	131.1		129.2	139.6	133.2	130.9
	...	...	...		...	...	...	...

accuracy by the expression  $\frac{1}{2} \{ \frac{1}{2} (z_1 + z_2) + z_3 \}$ , instead of the mean  $\frac{1}{3} (z_1 + z_2 + z_3)$ . The variances of these two expressions being in the ratio  $\frac{2}{3} : \frac{1}{3}$  or  $\frac{2}{3}$ , the amount of information lost will be  $\frac{1}{3}$ , which is not likely to be serious unless the variety involved turns out to be of special interest. If there are six replications, instead of four, the corresponding ratio is  $\frac{5}{24} : \frac{1}{3}$  or  $\frac{5}{8}$ .

Table IX shows the sum of the two replicates of each variety for each grouping, arranged in the same manner as Table I. In order to save

space only the values for varieties having  $v=1$  or 2 are included. The sum of these two tables is also shown, together with the quantities  $\frac{1}{2}(\bar{y}_{u.}-\bar{x}_{u.})$  and  $\frac{1}{2}(\bar{x}_{.v}-\bar{y}_{.v})$ , the first of the former of these quantities,  $-10.7$ , for example, being equal to  $\frac{1}{28}(291-590)$ . From these values the adjusted values of the varietal means are derived. These are shown in the last line of Table IX, that for variety 11, for example, being given by

$$\frac{1}{4}.102 - 10.7 - 1.3 + 100 = 113.5.$$

The analysis of variance is shown in Table X. The sum of squares for (mean + groups + sets + varieties) is equal to

$$142,788 + 6299.86 + 5370.71 - 208.18 = 154,250.39,$$

the terms being given in the same order as in the formula of section IV. The sum of squares for (mean + groups + sets) is 147,149.71. The difference gives the sum of squares for varieties, and the difference of the first quantity from the total sum of squares arising from all  $x_{uv}$  and  $y_{uv}$ , namely 158,077, gives the error between groups.

Table X. *Analysis of variance: 7 × 7 arrangement*

	Degrees of freedom	Sum of squares	Mean square
Blocks (including groups and sets)	27	27928.63	1034.39
Varieties	48	7100.68	147.93
Error: Between groups	36	3826.61	106.29
Within groups	81*	9144.15	112.89
Total	192*	48000.07	250.00

\* 3 deducted for missing yields.

The estimates of error between and within groups are about equal, as they should be. The combined error mean square, 110.86, may be compared with the treatment mean square, 147.93, by means of the  $z$  test. The value of  $z$  obtained is 0.1440, whereas the 5 per cent. point for these numbers of degrees of freedom is equal to 0.1913. The observed value (which is slightly inflated because of the method of estimating the missing plots) is thus well within the range that may be expected by chance.

The standard errors of the differences of the adjusted varietal means are as follows:

Two varieties in same set (row or column):

$$\sqrt{\frac{2 \times 110.86}{4}} (1 + \frac{1}{7}) = \pm 7.96.$$

Two varieties not in same set:

$$\sqrt{\frac{2 \times 110.86}{4}} (1 + \frac{2}{7}) = \pm 8.44.$$

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If one of the varieties has a missing yield the standard errors will be increased approximately in the square root of the ratio  $(\frac{1}{4} + \frac{3}{8}) : (\frac{1}{4} + \frac{1}{4})$  or  $\sqrt{5/4}$ , and if both the varieties have missing yields by  $\sqrt{3/2}$ .

In order to compare the accuracy of this pseudo-factorial arrangement with an arrangement in randomised blocks the pooled estimate of error from varieties and error should be taken. This is 121.64. The corresponding error for randomised blocks of 49 plots is 192.16. The ratio 192.16 : 121.64 = 1.580, multiplied by the factor  $(p+1)/(p+3)$  or 8/10, gives 1.264, so that the pseudo-factorial arrangement is 26 per cent. more efficient. If a  $7 \times 7$  Latin-square pseudo-factorial arrangement had been adopted (this would require a multiple of 3 replications) the factor would be  $(p+1)/(p+2\frac{1}{2})$  or 16/19, giving the ratio 1.331, *i.e.* a gain of a further 7 per cent.

Table XI. *Arrangement of blocks and varieties:  $4 \times 4 \times 4$  pseudo-factorial arrangement*

	M	L	K	J	I	H	G	F	E	D
2	342	421	424	234	134				142	*
4	242	423	422	334	434				144	*
6	142	341	442	231	223				143	*
8	442	311	432	431	224				141	211
10	213	321	422	331	221				243	213
12	313	331	412	131	222				143	214
14	413	112	222	124	143	233			343	212
16	113	132	232	114	133	243			443	432
18	323	122	242	144	113	213			111	132
20	333	142	212	134	123	223			113	232
22	343	244	441	114	131	414	342		114	332
24	313	*†	421	214	141	411	341		112	222
26	431	224	431	414	121	412	344			122
28	434	214	411	314	111	413	343			322
30	432	234	424	444	321	121	313			422
32	433	241	444	443	323	221	312	433		322
34	323	*†	434	441	324	421	314	423		312
36	423	231	414	442	322	321	311	413		332
38	223	211	244	324	433	121	224	443		342
40	123	221	242	344	233	124	424	334		232
42	412	312	241	334	133	122	124	333		231
44	212	112	243	314	333	*†	324	332		233
46						123	132	331		234
48						141	134	411		144
50						341	133	111		444
52						241	131	211		344
54						441	311	*†		244

\* Discarded.

† Yield missing.

Blocks of 64 plots: (1) M, L, K; (2) J, I, H; (3) G, F, E, D.

(c)  $4 \times 4 \times 4$  pseudo-factorial arrangement

For this arrangement the plots were divided without reference to the yields into 48 blocks of four plots each, and the 64 varieties assigned at random as in the  $7 \times 7$  arrangement. Missing plots were, however, excluded. The actual arrangement obtained is shown in Table XI.

The first step in the computations is to set out the yields in order as in Table XII, where the yields of the first group and the sum of all the groupings are shown for varieties having  $v=1$  or 2, together with their marginal totals. The yields of the other two groupings and of varieties having  $v=3$  or 4, have been omitted to save space.

The next step is to prepare a table of the quantities  $c_{vw}$ ,  $c_{u,v}$  and  $c_{uv}$ . These are also shown in Table XII. Thus

$$-3.38 = \frac{1}{24} 365 - \frac{1}{8} 156 - \frac{1}{96} 1178 + \frac{1}{32} 322.$$

Finally a table of the adjusted yields may be prepared by adding these corrections to one-third of the sum of all the groupings. Thus

$$132.16 = 100 + \frac{1}{3} (84) - 3.38 + 6.03 + 1.51.$$

The analysis of variance is shown in Table XIII. The sum of squares due to treatments is given by the sum of the products of the adjusted values and the variety totals of Table XII, less the sum of the products of the block totals and the corresponding marginal means of the adjusted values,  $\bar{t}_{vw}$ , etc. (not shown). Omitting the 100's from the  $t$ 's the first sum of products is  $130,560.81$ , while the second is given by the sum of  $27.98 \times 156$ , etc., and two similar sums for  $y$  and  $z$ , which three together total  $124,189.42$ . The computation of the other sums of squares requires no comment.

The standard error of the difference of two varieties occurring in the same set (i.e. having two of the three numbers  $u$ ,  $v$ ,  $w$  the same) is

$$\sqrt{\frac{2 \times 100.69 \times 21}{48}} = \pm 9.39,$$

that of two varieties having one of the three numbers  $u$ ,  $v$ ,  $w$  the same is

$$\sqrt{\frac{100.69 \times 48}{48}} = \pm 10.03,$$

and that of two varieties having no common number is

$$\sqrt{\frac{100.69 \times 50}{48}} = \pm 10.24.$$

To compare the accuracy of the  $4 \times 4 \times 4$  arrangement with an arrangement in ordinary randomised blocks of 64 plots the pooled residual

Table XII.  $4 \times 4 \times 4$  arrangement

Yields of first group ( $c_{uvw}$ )															
1				2				3				4			
u	v	w	u.v.w	1	2	3	4	1	2	3	4	1	2	3	4
1	31	49	36	40	156	26	7	28	14	75	103	37	19	32	10
2	53	29	43	39	164	30	21	20	19	35	95	55	48	44	33
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
141	91	123	108	403	463	72	28	55	36	191	...	339	122	78	102
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Sum of three groups ( $3m_{uvw}$ )															
1	84	123	75	83	365	70	72	89	51	282	36	58	45	69	208
2	112	89	108	63	372	87	71	105	71	334	72	91	38	69	270
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
305	304	324	242	1355	200	241	359	171	1031	271	370	232	316	1189	362
Corrections ( $c_{uvw}, c_{u.w}, c_{v.w}$ )															
+3.381				+5.97				+6.91				+10.451			
-6.98				+1.18				+3.66				-2.61			
...				...				...				...			
+6.03				-0.26				+9.95				+1.74			
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variance of varieties and error, 100.89, must be multiplied by the factor  $\frac{23}{42}$ , giving 151.34. The ratio of the residual variance of blocks of 64 plots, 219.15, to this is 1.448, so that the pseudo-factorial arrangement is 45 per cent. more efficient.

Table XIII. *Analysis of variance:  $4 \times 4 \times 4$  arrangement*

	Degrees of freedom	Sum of squares	Mean square
Blocks (groups and sets)	47	33004.74	702.23
Varieties	63	6371.39	101.13
Error between groups	81	8155.86	100.69
Total	191	47531.99	248.86

If instead of a  $4 \times 4 \times 4$  arrangement an  $8 \times 8$  arrangement had been adopted (an even number of replications would be required for this), the residual variance of blocks of eight plots, 119.51, would have to be multiplied by the factor  $\frac{11}{9}$ , giving a ratio of 1.500. With an  $8 \times 8$  Latin-square pseudo-factorial arrangement the factor would be  $\frac{21}{18}$ , giving a ratio of 1.572.

#### X. COMPARISON OF THE RELATIVE EFFICIENCIES OF THE DESIGNS OF SECTION IX

A summary of the efficiencies of the various arrangements discussed in the last section, relative to that of ordinary randomised blocks, is given in Table XIV. The efficiencies in the limiting case in which the error variance is the same for all block sizes, *i.e.* when there is no association between neighbouring plots, are also given for comparison.

The pseudo-factorial arrangements have proved decidedly the most efficient in every case, more especially with 64 varieties. Even the  $4 \times 4 \times 4$  arrangement, which was really only included to illustrate the computations, has been very successful, though for only 64 varieties and a small number of replications the  $8 \times 8$  arrangements are to be preferred. Three-dimensional arrangements are likely to be most advantageous with a larger number of varieties, and in cases in which there are sufficient replications for the randomised blocks to be replaced by Latin squares.

It is not claimed that the uniformity trial on which the examples are based is necessarily typical of the soil heterogeneity ordinarily met with. It was in fact the first uniformity trial encountered in a search of the literature which contained the requisite number of plots of reasonable size. A certain number of trials were inspected which contained a large

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number of small plots ( $\frac{1}{500} - \frac{1}{200}$  acre), and none of these exhibited such a large degree of soil heterogeneity. It may be doubted, however, whether trials of this nature fairly represent the type of land ordinarily available for experiment: in some cases, at least, it would seem that a specially uniform piece of land was chosen, often after inspection of the growing crop.

In any case the values of Table XIV for the case where there is no association between plots show that the pseudo-factorial arrangements

Table XIV. *Comparison of various types of arrangement. (Efficiencies expressed as a percentage of that of ordinary randomised blocks)*

	Chosen example	No association between plots
A. 49 varieties		
Randomised blocks of 49 plots	100	100
Blocks of 7 plots with 2 controls per block	84.0	53.2
Systematic controls every 5th or 6th plot (differences)	98.0	47.7
Systematic controls every 5th or 6th plot (covariance)	103.5	76.4
7 × 7 pseudo-factorial arrangement	126.4	80
7 × 7 Latin-square pseudo-factorial arrangement	133.1	84.2
B. 64 varieties		
Randomised blocks of 64 plots	100	100
Blocks of 8 plots with 2 controls per block	100.3	54.7
Systematic controls every 5th or 6th plot (differences)	111.9	47.7
Systematic controls every 5th or 6th plot (covariance)	118.0	76.4
4 × 4 × 4 pseudo-factorial arrangement	144.8	66.7
8 × 8 pseudo-factorial arrangement	150.0	81.8
8 × 8 Latin-square pseudo-factorial arrangement	157.2	85.7

are never likely to be much less efficient than systematic controls, and are always decidedly more efficient than random controls. They may be somewhat less efficient than ordinary randomised blocks when there is little soil heterogeneity to eliminate.

## XI. SUMMARY

A new method of arranging variety trials involving large numbers of varieties is described. This type of arrangement, for which the name *pseudo-factorial* arrangement is proposed, enables the block size to be kept small without the use of controls.

Various possible types of pseudo-factorial arrangement are discussed in detail and the necessary formulae developed. The appropriate methods of computation are illustrated by numerical examples based on the results of a uniformity trial on orange trees. It is shown that pseudo-factorial arrangements are likely to be more efficient than arrangements

involving the use of controls. In cases where there is considerable soil heterogeneity they are also markedly more efficient than randomised blocks containing all the varieties. In the chosen example gains in efficiency ranging from 26 to 57 per cent. were obtained.

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# AN EXAMINATION OF THE SAMPLING OBSERVATIONS ON WHEAT OF THE CROP-WEATHER SCHEME

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(With Five Text-figures)

## I. THE SAMPLING OBSERVATIONS ON WHEAT OF THE CROP-WEATHER SCHEME

IN 1922, a committee was formed of representatives from the Ministry of Agriculture and Fisheries, the Department of Agriculture for Scotland, and the Meteorological Office, in order to draw up a scheme whose aim should be the collection of observations both on the growth of various cereal, root and fruit crops, and also on the meteorological conditions experienced by the agricultural experimental stations concerned. It was hoped that these two sets of observations would ultimately provide material whereby the reactions of the crops to weather conditions might be studied. The scheme was not successful, and J. O. Irwin, reviewing it in 1929<sup>(1)</sup>, stated that the agricultural observations were inadequate. The only results which emerged were those connected with the length of the germination interval, which, in the case of spring and winter oats, spring barley, and winter wheat, he found to be significantly correlated with the mean soil temperature during the interval<sup>(2)</sup>.

In 1928 a new scheme was evolved which set out a series of quantitative observations appropriate to each of the three crops, wheat, barley and sugar beet. The considerable time and labour involved prevented the scheme from materialising so far as the two latter crops were concerned, but observations on wheat were begun immediately at three agricultural stations. Subsequently the scheme was modified and improved in various ways, while the stations participating changed from year to year, until, in 1932, the design was completely revised and the current Sampling Observations on Wheat initiated. These observations have furnished the material for the present paper. They are at present carried out at each of ten experimental stations, ranging from Seale Hayne in

Devon to Boghall in Scotland, and they provide a record of plant number, shoot number and shoot height, ear number and ear height, at the appropriate times of year for the wheat crops grown at each of these stations. The interval between the successive observations varies from 1 day at the time when the crop appears above ground to 3 weeks during certain of the winter months. Thus full information may be obtained concerning the following stages in the crop's progress:

- (1) Appearance above ground,
- (2) Tillering,
- (3) Attainment of maximum shoot number,
- (4) Maximum rate of increase in shoot height,
- (5) Ear emergence,
- (6) Fitness for cutting,

these stages being defined according to arbitrary conventions wherever necessary.

All the stations take their observations on each of two standard varieties, Squarehead's Master and Yeoman, and, in addition, some grow a third variety which is considered to be particularly suited to local conditions.

The seed for the two standard varieties is supplied by the National Institute of Agricultural Botany, the rate of sowing being fixed at  $2\frac{1}{4}$  bushels per acre for all English stations and 3 bushels per acre in Scotland.

The main part of the experimental area is laid out in eight blocks, each 25 yards long, and either two or three drill widths wide, the varieties being assigned to the strips at random within each block. Each strip is split in half lengthways by stopping the centre coulter of the drill, each half-strip being then six rows wide. Additional areas of 2 sq. m. are marked out at either end of each half-strip and are used for the observations on appearance above ground. A variety is said to have appeared above ground in any one of these areas when twenty plants have appeared. The mean of the thirty-two dates so obtained gives the date of appearance for the variety.

All other observations are taken by sampling the main area. The sampling unit consists of  $\frac{1}{4}$  m. of each of the four central rows of the half-drill strip, the two outermost rows of the six being omitted. Each sampling unit therefore contains 1 m. of drill row, and two such units are taken from each half-strip so that 32 m. of each variety are sampled at every observation. The position within a half-strip of each sampling unit taken

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is determined according to random numbers previously arranged in the laboratory.

Plant number counts for each variety are begun about 3 weeks after the variety has appeared above ground and are continued at 3-weekly intervals until the commencement of tillering, when weekly counts of both shoot and plant numbers commence. By this means the date of tillering, which is defined as that on which the ratio of shoots to plants passes the value 2, can be determined. Soon after tillering it becomes impossible to continue the plant counts owing to the difficulty of distinguishing individual plants, but the shoot counts are continued at fortnightly intervals until shortly before the crop is harvested.

As soon as the shoots begin to grow appreciably, measurements of shoot height are taken on the shoots nearest the two ends of the four rows of the sampling units. Thus each unit supplies eight measurements. The height is measured to the top of the sheaf of the youngest fully expanded leaf. When the ears begin to emerge ear heights (measured to the awn of the highest flower) are taken in addition. For a short period counts of emerged and non-emerged ears are necessary at 2-day intervals in order to fix the date of ear emergence, which is defined as that on which 50 per cent. of the ears have emerged. Two counts on either side are sufficient to determine this date.

The final observations taken are the yields of grain and straw, the samples from all the stations being sent to Rothamsted and thrashed there.

If the scheme is carried out for even as short a period as 10 years, it should, in conjunction with the meteorological observations taken at each of the stations concerned, give a unique set of data as to the influence of weather conditions on the chief stages in the wheat crop's growth, from germination to maturity. It will also provide data which should show to what extent the successive stages are connected with one another, and hence whether more reliable yield predictions can be obtained either from the earlier measurements of the crop's own growth alone or from these in conjunction with meteorological observations, rather than from a consideration of the latter alone. This point has been investigated so far as is at present possible, but in view of the importance of the problem, and the small amount of data available, it is considered best to withhold a full discussion of the conclusions reached until a further year's data are to hand. A brief account of the results obtained thus far has been published in the *Journal of the Ministry of Agriculture* (3).

It is the aim of this paper to give an account of the connections found

to exist between the first four stages of the crop's growth, as set out above, with various meteorological factors which have been selected from all those available according to general considerations of relevance. It was not possible to deal with all the points on which the sampling observations on wheat furnish information, and ear emergence and the date of fitness for cutting have been left for future investigations. Since only 3 years' observations are at present available—the results from previous years being too fragmentary to be of use—it is to be regarded as a preliminary report.

The observers' records, together with the summaries of them which are published quarterly in the *Journal of the Ministry of Agriculture*, have provided the necessary agricultural data. The meteorological data have been obtained from the standard records sheets kept by each station. The observers' records are periodically sent to Rothamsted and filed there for future reference, while access may be had to the meteorological data at the Meteorological Office in London.

With one exception, differences between the varieties of wheat have not been considered, and the results given are based on the mean values for the two standard ones.

## II. GRAPHICAL REPRESENTATION OF THE WHEAT CROP'S GROWTH

From the observers' records it is possible to draw mean curves giving a general idea of the successive stages in the crop's progress for each of the 3 years 1932-3, 1933-4, 1934-5. These are shown in Fig. 1.

Every point on each of the shoot and plant number curves is the mean of five observations, these observations being those for the mean

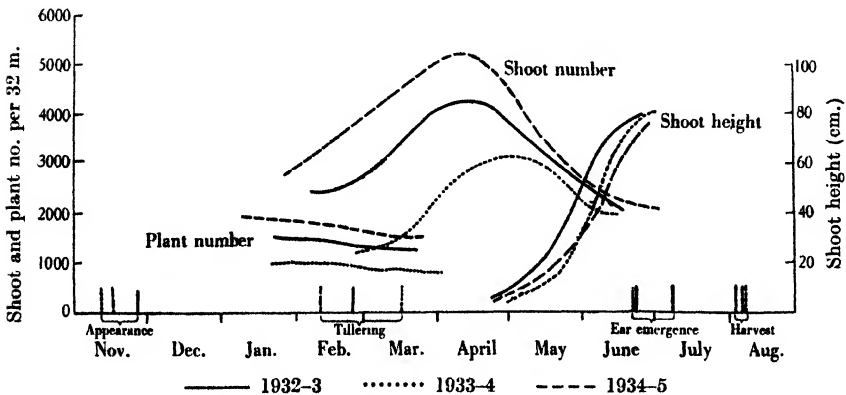


Fig. 1. Mean growth curves for the 3 years 1932-3, 1933-4, 1934-5.

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of the two standard varieties grown at five stations, taken at corresponding stages of the crop's development. To obtain corresponding points it was first necessary to draw individual shoot- and plant-number curves for each variety at each station in all 3 years. For each year the average period from appearance above ground to harvest was then determined, and divided, for convenience, into thirty equal intervals. The similar periods for the individual stations were divided into the same number of intervals, the number of days in each interval being different for each station. Readings at the beginning of the first, second, ... interval were then corresponding observations and their mean value was

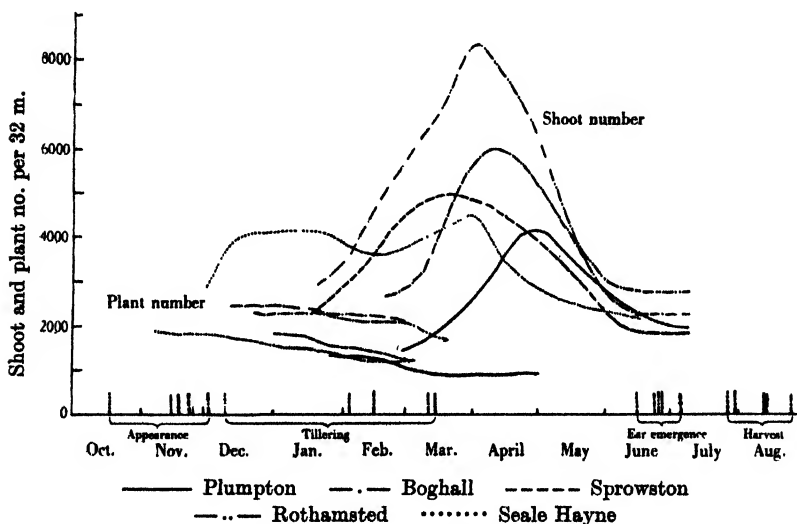


Fig. 2 a. Growth curves for five stations in 1934-5. (Shoot and plant number.)

plotted at the beginning of the first, second, ... interval of the average period, thus giving the mean curve for the year considered.

The stations used to obtain the 1932-3 and the 1933-4 curves were Seale Hayne, Rothamsted, Newport, Boghall and Sprowston, these being the stations with the most complete records. In the 1934-5 curve Plumpton was substituted for Newport, since many observations had been missed at the latter station.

The shoot-height curves were obtained in exactly the same way, except that the Seale Hayne readings had to be omitted, so that each point represents the mean of four observations.

The three sets of curves all show the same main features: the increase in the shoot number to a maximum in spring, the subsequent rapid

decrease as the shoots begin to grow in height, and the rapid increase in shoot height which lasts until ear emergence, after which very little increase takes place.

In addition several other points are worthy of notice; the most striking of these is the similarity of the shoot-number curves after the point of maximum shoot number has been passed. The final shoot number is close to 2000 in all three years, although the first plant counts vary from about 1000 in 1933-4, with a maximum shoot number of approximately

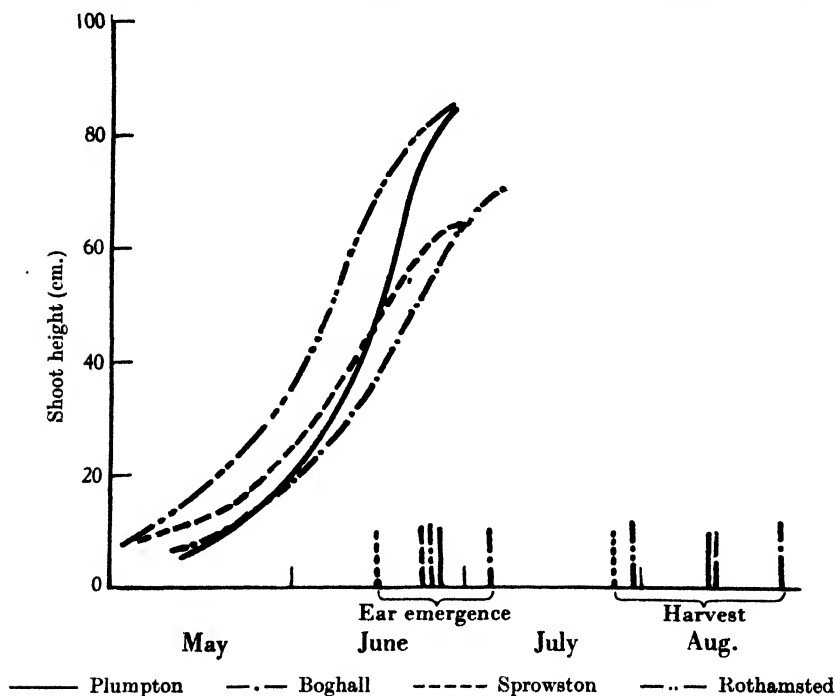


Fig. 2 b. Growth curves for four stations in 1934-5. (Shoot height.)

3000, to about 2000 in 1934-5, with a maximum shoot number of over 5000. This suggests that there is a small range of optimum final shoot numbers which suits the development of the wheat best; and that this is attained by the dying off of a larger or smaller number of shoots in the spring and early summer, according as the maximum shoot number was large or small.

Secondly, the mean sowing dates for the 3 years (which are not shown in the figure) are very close together, only varying between October 28 and 31. The mean date of appearance above ground varies from Novem-

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ber 11 to 25, the mean tillering date from February 11 to March 18, and the mean date of ear emergence from June 23 to July 9. With these wide divergences during the growing season, it is somewhat surprising that the greatest difference between the mean harvest dates is only 2 days, and it will be of interest to notice whether this phenomenon is repeated in subsequent years.

It will be seen that the shoot-height curves are all very similar and show the slackening off of growth at the time of ear emergence.

Fig. 2 (*a* and *b*) illustrates the amount of variation which may exist between individual stations in any one year. The 1934-5 curves, for the five stations used in obtaining the mean curve for this year, are given. The variation between them is great, and the individual values are more influenced by errors in the observations than are the mean curves, but they show the same main features.

The similarity of the shoot-number curves, after the time of maximum shoot number has been passed, is apparent, and although in the case of Seale Hayne the crop appears to have suffered a check in February and March, the final shoot number is still approximately 2000; while at Rothamsted, although the maximum shoot number is unusually high, the subsequent dying off of shoots is so rapid that the latter part of the shoot-number curve conforms very well with those from the other stations. In this year the range of variation in sowing date for these stations was about 5 weeks; that in the date of appearance above ground about 6, in tillering date 12, in ear emergence 3, and in harvest about  $3\frac{1}{2}$  weeks.

### III. STATISTICAL ANALYSIS OF THE DATA

It has been stated that ten agricultural stations agreed to take part in the scheme. Of these Plumpton and Cirencester entered a year late, and in the case of Cirencester many of the observations in the next 2 years were missing, so that it was considered advisable to omit returns from this station. With this omission records from eight stations for 3 years, and from one, Plumpton, for 2 years, were available. In several of these records individual observations were unfortunately missing, as will be found in following through the paper.

The data relevant to each stage of growth investigated were analysed by performing analyses of variance and co-variance on the factors considered, eliminating variations due to differences between years and between places. Partial regression equations could then be obtained from the residual sums of squares and products. All the partial regression

coefficients given are therefore based on residual deviations both from place and year means.

Before carrying out the analyses, the fact had to be considered that there was at least one observation missing from the data concerned with each stage of growth, excepting that dealing with appearance above ground. F. Yates has given a simple method of estimating such values in an ordinary analysis of variance in which it is desired to remove block and treatment effects (4). The method can be readily extended to analyses of covariance, and used to estimate missing values in both the dependent and independent variables. The values obtained for the regression coefficients, and hence for the residual sum of squares after fitting the independent variables, when the estimated values are included so that the analyses can be carried out as if the completed data formed an orthogonal set, are the same as those obtained by fitting constants to the incomplete data. In practice it is simplest to estimate the missing value and proceed according to the first method.

One further complication is introduced by the lack of orthogonality of the data. The usual tests of significance of the differences between places and between years, after eliminating the variation associated with the independent variables considered (5), are no longer quite exact. The modifications necessary to obtain exact tests are demonstrated in the paper referred to above (4) for the case of a single analysis of variance. The method can be extended to analyses of covariance, and the exact sum of squares, needed for testing the significance of the differences required, found. The modification has not been employed in this paper, since it was thought that the resulting change in the conclusions would be slight. The tests of significance are therefore applied as though the completed data formed an orthogonal set.

It is essential that both place and year effects should be eliminated, since otherwise misleading results might be obtained. For example, in all three years the highest temperatures prevail at Seale Hayne. In addition it is one of the stations which sow earliest each year. If differences between places were not removed, a significant regression of sowing date on temperature at the time of sowing might appear. The validity of such a result would be questionable, until, in addition, it was seen whether there was a significant regression of sowing date on temperature at each station.

The same procedure also eliminates various possible sources of disturbance. For example, if it had happened that the seed supplied to the stations was more productive in one year than in the other two, the



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effects on the regression coefficients of the resulting variation in the yields, if such existed, would be small after differences between the mean yields from year to year had been removed in the analysis. It should be noticed that the existence of such an uninvestigated factor would not, in general, pass unnoticed if the difference between the yields from year to year were significant, for these differences would remain significant after the effects of the factors investigated were eliminated from them.

In the same way differences between the individual characteristics of the stations, provided that they are consistently operative throughout the 3-year period, are eliminated when differences between places are removed, and the regressions based on residual deviations from the mean values. Differences in soil, in altitude, and, to a large extent, in methods of cultivation, come under this category.

### IV. INTERVAL FROM SOWING TO APPEARANCE ABOVE GROUND

The results of this section of the work were more clear-cut than those connected with any of the other three growth stages investigated. This is to be expected, since the period concerned is very short and the meteorological factors which influence the crop are likely to be less varied than at any other stage of its growth. The date of appearance is usually well determined by the observers according to the method described in Section I.

The Plumpton observations were omitted, since Plumpton did not begin to keep meteorological records until February 1934. This left eight stations with 3 years' observations at each. It seemed probable that the temperature would be the most influential factor in determining the length of the interval, but since it was possible that either the rainfall or the variability of the temperature might exert some influence, the first regression to be tried was that of length of interval on mean soil temperature, variance of temperature, and rainfall during the interval—the soil temperature used being that at 4 in. depth.

Firstly with regard to mean soil temperature; every station records the soil temperature at 9 a.m. each day. Since it was more convenient to work with the mean daily temperature some method of estimating this from the 9 a.m. reading was required. At Rothamsted, daily readings had been taken at 9 a.m., 3 p.m. and 9 p.m. throughout the years 1930, 1931, 1932 and 1933. The difference between the 9 a.m. and the mean temperature was taken as constant over each month, and whatever differences were found to exist at Rothamsted were used at each of the other stations.

The first step was to compute the mean 9 a.m., the mean 3 p.m. and the mean 9 p.m. temperature for each month of each of the 4 years. Then for each month the mean daily temperature can be estimated by fitting a sine curve to these mean values. It is at once apparent that the 3 p.m. mean is irrelevant, and the mean temperature for the month is simply the mean of the 9 a.m. and 9 p.m. readings. The difference between this mean and the 9 a.m. mean (*i.e.* one-half the difference of the 9 p.m. and 9 a.m. means) gives the appropriate correction for each month. Table I shows these differences.

Table I. *Differences between 9 a.m. soil temperature and mean soil temperature (° F.)*

Month... Year	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1930	0.5	0.6	1.5	1.4	1.3	2.4	1.2	1.6	0.9	0.7	0.7	0.5
1931	0.6	0.9	1.0	1.4	1.6	1.8	1.4	1.6	1.2	1.3	0.9	0.3
1932	0.6	0.6	1.3	1.0	1.1	2.2	1.3	1.9	1.1	0.5	0.6	0.3
1933	0.1	0.5	1.9	1.7	1.9	2.0	2.1	2.3	1.5	0.7	0.3	0.3
Mean	0.5	0.7	1.4	1.4	1.5	2.1	1.5	1.9	1.2	0.8	0.6	0.4
Fitted mean	0.52	0.76	1.11	1.47	1.75	1.87	1.81	1.57	1.23	0.87	0.58	0.46

The sine curve  $Y = 1.167 + 0.1236 \sin x - 0.7064 \cos x$ , where  $x$  takes the values  $\frac{\pi}{12}$ ,  $\frac{3\pi}{12}$ , ...,  $\frac{23\pi}{12}$ , was fitted to the twelve mean values given in the fifth line of the table, the coefficients having been estimated by the method of least squares. The fitted values,  $Y$ , are shown in the last line of the table.

The residual variance in the means, after fitting, is 0.188 (in units of a single observation). This is just significantly above the residual variance of the individual observations of Table I (0.084), after removing differences due to months and years. The irregularities indicated by this significance do not appear to follow any consistent law, indeed if allowance were made for the fact that there is a greater variation from year to year in the summer than in the winter months, the verdict of significance would probably not be made. The differences between years are insignificant.

The values  $Y$  were used as differences to be added to the 9 a.m. readings at all stations in those months of the years 1932, 1933 and 1934 for which they were required. The mean soil temperature, and its variance, over the interval proper to each station in each year could therefore be immediately obtained.

Secondly with regard to rainfall. It was reasonable to suppose that

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the amount of moisture present in the soil, rather than the actual amount of rain precipitated, would influence the rate of growth of the crop. No record of soil moisture was available, and the rainfall present in an ordinary gauge is a poor measure of it. However, by taking into account the daily rainfall for a short period before the date of sowing and weighting this in accordance with its nearness to the sowing date, it was hoped to arrive at a somewhat more satisfactory estimate. A 12-day period was chosen for convenience, and the daily rainfall therein given weights of  $\frac{1}{10}, \frac{2}{10}, \dots, \frac{9}{10}, 1, 1, 1$ , and added to the total rainfall in the interval, the rainfall on the final day being given half-weight. This, when divided by the appropriate divisor—for a period of 21 days, the divisor would be  $7.5 + 20 + 0.5$ —gave the weighted rainfall variate used in the analysis.

From the results of the preliminary analysis involving mean soil temperature, variance of temperature, and weighted rainfall, it appeared that neither of the partial effects of rainfall and variance of temperature were of any consequence in determining the length of the interval below ground, the probabilities of their occurring by chance being between 0.4 and 0.5. On the other hand, mean soil temperature appeared to be highly significant, the probability of its occurrence by chance being less than 0.01. For this reason, a second analysis was carried out, from which rainfall and variance of temperature were omitted, but (mean soil temperature)<sup>2</sup> was introduced in order to determine whether the relation between interval and temperature was better represented by a quadratic law.

Table II. *The lengths of the intervals below ground (*t*) in days*

Station ...	Boghall	Woburn	Seale Hayne	Roth- amsted	Wye	Sprow- ston	Long Sutton	Newport
Year								
1932-3	36.7	11.0	10.0	15.4	20.3	32.0	26.5	33.0
1933-4	36.0	36.4	19.2	18.7	22.8	23.4	25.0	27.0
1934-5	19.4	20.7	9.3	21.7	17.8	19.0	9.8	21.5

Mean value 22.19 days

Table III. *The values of the mean soil temperature *T* (° F.)*

Station ...	Boghall	Woburn	Seale Hayne	Roth- amsted	Wye	Sprow- ston	Long Sutton	Newport
Year								
1932-3	37.63	49.32	52.06	46.01	45.71	42.29	40.89	41.49
1933-4	39.01	38.50	45.97	44.48	46.10	44.23	42.94	42.47
1934-5	42.36	42.35	52.02	41.09	46.41	44.21	51.57	41.61

Mean value 44.20° F.

Table II gives the values of  $t$ , the length of the interval in days, and Table III the corresponding value of  $T$ , the mean soil temperature in the interval. The relation between these variables is shown graphically in Fig. 3, which also shows the approximate curve which was finally fitted.

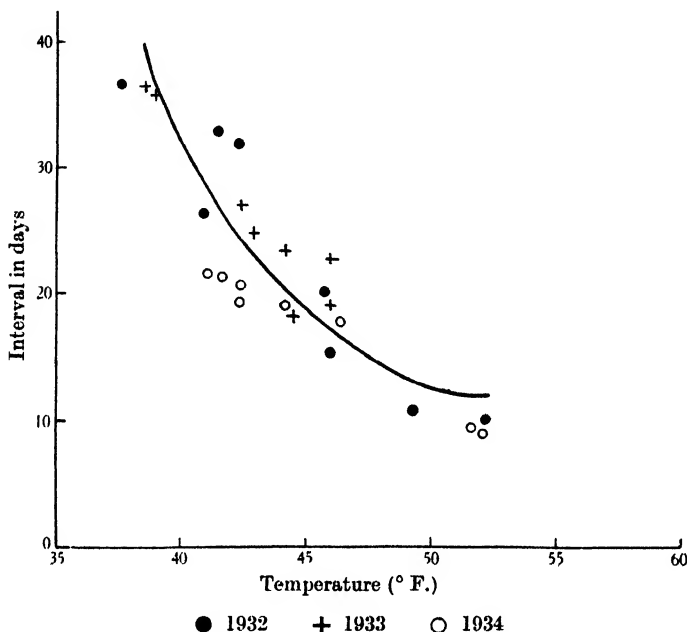


Fig. 3. The relation between the interval below ground and the mean soil temperature.

The complete analysis of the covariance of  $t$ ,  $T$  and  $T^2$  is shown in Table IV. The variation in the intervals due to year effects nearly reaches the 0.05 level of significance, while the place effect is insignificant.

Table IV. *Analysis of covariance of  $t$ ,  $T$  and  $T^2$*

Variance due to	D.F.	$[t^2]$	$[T^2]$	$[(T^2)^2]/10^4$	$[t.T]$	$[t.T^2]/10^2$	$[T.T^2]/10^2$
Places	7	636.09	196.25	160.63	-332.61	-300.18	177.35
Years	2	308.56	20.70	17.88	-74.85	-68.83	19.23
Residue	14	628.45	156.09	127.97	-279.34	-247.62	141.11
Total	23	1573.10	373.04	306.48	-686.80	-616.63	337.69

As was stated in Section III, the regression coefficients are based on the residual deviations from the appropriate place and year means; that is, on the sums of squares and products given in the third line of the table.

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The interval  $t'$  to be expected at any station  $s$  in year  $y$  when the mean temperature is  $T^\circ$  F. is therefore

$$t'_{sy} = \bar{t}_{sy} + b (T_{sy} - \bar{T}_{sy}) + c (T_{sy}^2 - (\bar{T}^2)_{sy}),$$

where

$$\bar{t}_{sy} = \bar{t} + (\bar{t}_s - \bar{t}) + (\bar{t}_y - \bar{t}),$$

$$\bar{T}_{sy} = \bar{T} + (\bar{T}_s - \bar{T}) + (\bar{T}_y - \bar{T}),$$

$$(\bar{T}^2)_{sy} = \bar{T}^2 + (\bar{T}_s^2 - \bar{T}^2) + (\bar{T}_y^2 - \bar{T}^2),$$

$\bar{t}_s$ ,  $\bar{t}_y$ , and  $\bar{t}$  being the respective mean values of  $t$  taken over all years at station  $s$ , over all stations in year  $y$ , and over all years and stations, etc., and  $b$  and  $c$  the partial regression coefficients of the interval on mean soil temperature and (mean soil temperature)<sup>2</sup>.

The approximate form of this expression, obtained by ignoring differences between years and between places, *i.e.* by replacing  $\bar{t}_{sy}$  by  $\bar{t}$ , etc., and substituting the numerical values, is

$$t' = 350.32 - 12.90T + 0.1229T^2, \quad \dots\dots(1)$$

giving the approximate interval to be expected at any station in any year when the mean soil temperature is  $T$ . This curve is plotted in Fig. 3.

The standard errors of the regression coefficients are  $\pm 3.40$  and  $\pm 0.0376$  respectively. The effect of fitting the quadratic term, after variations associated with the linear one have been removed, is therefore significant, since it would occur by chance less than once in one hundred times. The complete analysis of regression is shown in Table V.

Table V. *Analysis of regression effects*

Variance due to	D.F.	Mean square
Mean soil temp.	1	499.92
(Mean soil temp.) <sup>2</sup>	1	60.51
Residue	12	5.67
Total	14	44.89

It can be seen from this table that if only the linear term were fitted the residual variance would be 9.89, *i.e.* the total variance would be reduced by 80 per cent. approximately, whereas, if in addition the square term is fitted the residual variance is 5.67 and the total variance reduced by about 87 per cent.

It remains to be seen whether this temperature effect accounts for differences in germination interval from year to year, which, as was found previously, border on significance; that is to say, whether the difference between the year means becomes insignificant when the temperature

effect has been eliminated. The method of testing this point has been described by Fisher (5). The necessary reduced analysis shows that the difference reaches the 0.05 level of significance. This fact must be accounted for by the action of factors influencing the germination interval at every station in the same way in any one year. One possible factor is the quality of the seed supplied by the Ministry. The mean germination interval was longest in 1933-4, and in this year the seed is known to have been badly infected with smut.

A similar reduced analysis for testing the significance of the differences between places when temperature effects are eliminated shows the judgment of non-significance formed on the original data to remain unaltered.

A study of Fig. 3 shows that the significance of the quadratic effect might be largely due to the differences between the years, and that the relationship might be better expressed by three linear regressions, each appropriate to one of the three years. This, however, did not prove to be the case. The residual mean square is nearly twice as great as it is when a quadratic regression is fitted, and on these grounds this method of expressing the temperature effect was rejected. The significant differences between the year means of the interval below ground are still not accounted for by temperature variations, when this method of fitting is used.

It will be seen from Fig. 3 that the approximate curve fits the plotted points quite well except at the upper end of the temperature range where the points fall consistently below it. This circumstance is to be explained by the fact that the curve was actually fitted to the residual deviations from both place and year means. The differences between the place means are insignificant, and so the positions of the points would be little affected if these differences were removed. On the other hand, the differences between the year means are significant, and it is of interest to replot the points when these differences are allowed for. This has been done in Fig. 4, where the points plotted have co-ordinates

$$t - (\bar{t}_y - \bar{t}), \quad T - (\bar{T}_y - \bar{T}),$$

where  $\bar{t}_y$  and  $\bar{T}_y$  are the mean values of the interval below ground and the mean temperature in this interval in year  $y$ , and  $\bar{t}$ ,  $\bar{T}$  are the corresponding general means. The 1934 points in particular receive a substantial vertical shift, and the points as a whole are more evenly distributed about the curve. In Fig. 5 the reciprocal curve has been plotted. Using the assumption that the plants have approximately the same distance to

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grow in order to reach the surface of the earth at all the stations, this curve gives the growth rate in arbitrary units over the range of temperatures supplied by the data. The equation of the curve may be written

$$\frac{1}{v} = c (350.32 - 12.90T + 0.1229T^2), \quad \dots\dots(2)$$

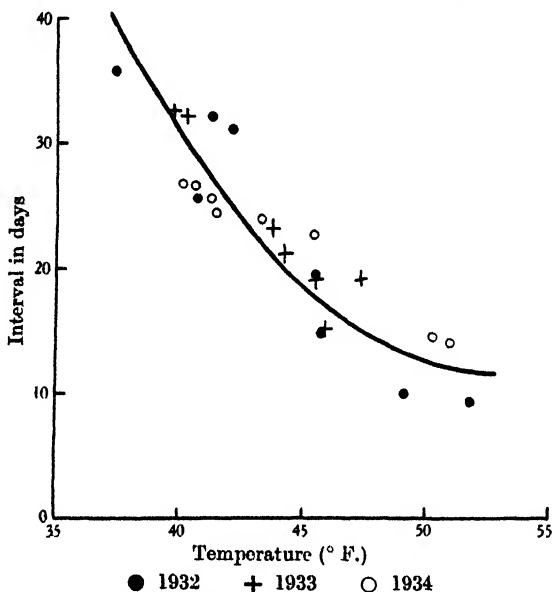


Fig. 4. Interval below ground and mean soil temperature, when differences between years have been eliminated.

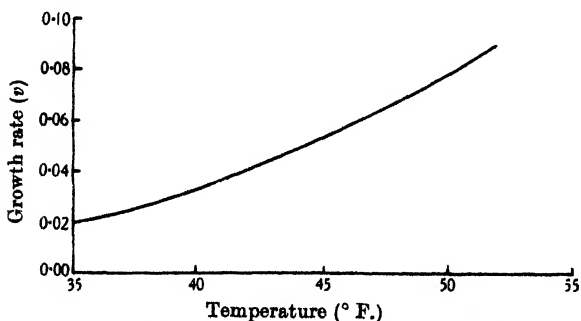


Fig. 5. Growth rate (arbitrary units) and soil temperature (° F.).

$c$  being an arbitrary constant. If the scale is fixed by taking the distance to the surface of the earth to be 1 in.,  $c = 1$ , and it gives the actual growth rate in inches per day.

It is of interest to see to what extent this expression for the growth rate conforms with Van 't Hoff's rule, which, when applied to plant physiology, states that the growth rate of a plant is doubled for each  $10^{\circ}\text{C.}$  rise within a normal range of temperatures. This law may be written algebraically

$$v = \lambda 2^{T/18}$$

(where  $T$  is in degrees F., and  $\lambda$  is an arbitrary constant), *i.e.*

$$\frac{1}{v} = \frac{1}{\lambda} \left( 1 - kT + \frac{k^2 T^2}{2} \right),$$

where

$$k = \frac{\log_e 2}{18} = 3.851 \times 10^{-2}.$$

The value of  $k$  as estimated from the first two terms of the expression for the growth rate obtained from the present data is  $3.682 \times 10^{-2}$  with a standard error of  $\pm 0.184 \times 10^{-2}$ . The two formulae are therefore in very good agreement. The estimate of  $k$  from the first and third terms of the formula is very much less reliable, since the linear term carries the main temperature effect, but it gives  $k = 2.650 \times 10^{-2}$ , a value which is at least not widely removed from that given by the Van 't Hoff rule.

Finally, one further point in connection with the interval below ground was investigated, namely, whether the difference between the intervals for the two varieties of wheat grown at each station is substantially influenced by the soil temperature. Only the linear term was considered in this investigation, since if this had proved to have a significant effect the quadratic term could have been introduced subsequently. The analysis was similar to that described above except that it was based on the differences between the intervals for the two varieties grown at each station, instead of on their means, and also that certain readings had to be omitted. The dates of appearance above ground had not been observed with sufficient accuracy to allow the differences between varieties to be determined at Newport in any of the three years, at Woburn in 1932 and at Sprowston in 1934. In addition, in 1933, there was a particularly heavy frost at Woburn, so that the ground was frozen hard for a week at the time when the crop should normally have been appearing; the difference between the varieties for this year was therefore possibly anomalous, and was omitted. Since, as before, it was desired to eliminate place effects, it was necessary to omit the Woburn readings entirely. The one missing value for Sprowston was estimated from all available data. Table VI shows the differences for every station in each



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year. It will be seen that with one exception, the interval below ground is consistently shorter for Yeoman than it is for Squarehead's Master.

Table VI. *Differences in interval below ground in days (S.H.M. — Y.)*

Station ...	Long Sutton	Seale Hayne	Wye	Rotham- sted	Sprowston	Boghall
Year						
1932	1.38	1.56	0.53	0.62	-0.06	1.28
1933	0.15	0.31	0.62	0.19	1.06	1.94
1934	1.53	1.62	0.38	1.28	0.70*	0.50

\* Estimated value.

Mean value 0.87.

The analysis of the differences showed the regression coefficient of difference in interval on mean soil temperature to be 0.0353, the corresponding value of  $t$  being 1.032, with 9 degrees of freedom. Such a value would occur by chance about 35 times in 100, so that it does not appear that the lag in appearance above ground is significantly different at different temperatures. Neither the year nor place effects are significant.

### V. TILLERING

As was stated in Section I the date of tillering of a variety is arbitrarily fixed as that on which there are twice as many shoots as plants of the variety. This occurs between January and March at the majority of stations, but at stations such as Seale Hayne, where the crop is always comparatively well advanced, it may be as early as December, and at northerly stations as late as April. The rate is defined as the number of tillers formed per hundred plants per week at the time of tillering, and varies from approximately 10 to 55.

The tillering data is less complete than that connected with appearance above ground; unfortunately the observers have not always taken sufficient, or sufficiently well timed, observations, so that about one-fifth of the number of observations which should have been available have had to be omitted entirely from the analyses, while of those included a further 15 per cent. are not reliable. In a very few of these cases the difficulty is primarily due to the fact that the standard method of determining the date and rate, which works very well in the great majority of cases, is not entirely satisfactory. These cases occur when a variety is on the point of tillering and then for some reason suffers a sudden check, so that tillering is delayed and proceeds very slowly until, after a period varying from perhaps 10 days to 3 weeks, the growth again assumes its normal rapidity and the variety tillers immediately. In such a case, a date a day or two

later than that on which the check occurred gives a better estimate for use in conjunction with data from other stations in discussing previous weather conditions than does the strict date of tillering on which the ratio shoots : plants actually reaches the 2 : 1 point.

(1) *The date of tillering, and the interval from appearance above ground to tillering*

In these analyses, the Plumpton readings were omitted, since no meteorological observations were available at Plumpton before February 1934, and it was desired to test the effect of temperature as the factor most likely to influence the date. Actually the weather conditions at the nearest meteorological station would probably not have differed widely from those at Plumpton, and the observations taken at it might have been substituted.

The Woburn readings had also to be excluded. In 1932-3 shoot counts were not taken until after the crop had tillered. In this case estimates of both rate and date are given in the Ministry reports, but a study of the observers' records shows that these estimations cannot be used with any degree of confidence. It has already been mentioned (Section IV) that in 1933-4, Woburn suffered a very heavy frost just as the plants were about to appear above ground. Growth was entirely checked, the ground being frozen hard for some days. In these circumstances, since the date of appearance above ground was to be used as one of the variables in the analyses of covariance, it appeared best to omit this observation. There was then no alternative but to sacrifice the 1934-5 reading, since place differences were to be eliminated.

The remaining seven stations were used in the analyses, two estimations being necessary. At Long Sutton the crop was destroyed by wireworm during March in the 1933-4 season, and at Newport tillering was completely missed in 1934-5.

In four cases the dates of tillering used were not those given in the Ministry reports. The alterations made are shown in Table VII, the

Table VII. *Alterations in tillering dates*

Station	Date given in Ministry report	Date used in analysis	Date for corresponding unaltered variety
Long Sutton, 1932-3 (S.H.M.)	April 1.25	Mar. 24	Mar. 24.26 (Y.)
Rothamsted, 1932-3 (Y.)	Jan. 18.26	Jan. 31	Feb. 4.30 (S.H.M.)
Long Sutton, 1934-5 (S.H.M.)	Feb. 3.30	Jan. 15	Jan. 3.01 (Y.)
Wye, 1934-5 (S.H.M.)	Mar. 18.43	Feb. 10	—
Wye, 1934-5 (Y.)	Mar. 21.25	Feb. 10	—

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figures in the fourth column showing the tillering dates for the unaltered variety at the same station and year.

With the exception of the alteration in the Rothamsted date all the changes were made for the reasons set out in the second paragraph of this section. The Rothamsted alteration was made because it appeared from the graphs of the shoot and plant counts for this station that a doubtful shoot count made Yeoman appear to tiller before the real tillering stage had actually commenced. The corrected estimations were all made directly from the shoot and plant graphs of the stations concerned, and the justification for the changes lies simply in the fact that the date of tillering is an arbitrarily defined date aiming to show the time at which the crop has reached a certain stage of growth. If the complete graphs of the observers' records indicate that in a few cases this stage is reached at a time slightly different from that arrived at by the strict computation based on four (or sometimes three) observations only, it appears best to follow the former. It will be seen that in each case the altered date is very much closer to that given for the unaltered variety at the same station.

The date of tillering was investigated first, and the factor whose influence on it was considered initially was the mean temperature from the date of appearance above ground to tillering. The date of appearance above ground was also included in the analysis, since it is clear that if a crop appears above ground in early November, it is probable that the mean temperature from appearance above ground to tillering will be higher than if it had been delayed until late December. In fact, the correlation between the date of appearance and the succeeding mean temperature when year and place effects have been eliminated is  $-0.55$ , a correlation which would occur by chance alone in about 6 cases out of 100. Since it is probable that a close association exists between this date and the date of tillering, the effect of temperature after eliminating that due to variations in the date of appearance is required, as otherwise apparently significant temperature effects might appear which were in fact only due to the association between temperature and date of appearance.

Tables VIII A, B and C show the dates of tillering, the dates of appearance above ground, and the mean temperatures in the interval, respectively. Both sets of dates are measured from February 1, as a convenient arbitrary origin.

The analysis of covariance is shown in Table IX. Both the year and the place effects are significant, each having a probability of occurrence by chance ( $P$ ) which is less than 0.01.

Table VIII A. *Tillering dates, measured from February 1*

Station ... Year	Long Sutton	Boghall	Newport	Sprow- ston	Wye	Roth- amsted	Seale Hayne
1932-3	51	55	46	48	33	1	- 36
1933-4	47	56	54	59	56	57	0
1934-5	- 23	39	13*	3	9	14	- 52

\* Estimated value.

Mean value 25.3.

Table VIII B. *Dates of appearance above ground, measured from February 1*

Station ... Year	Long Sutton	Boghall	Newport	Sprow- ston	Wye	Roth- amsted	Seale Hayne
1932-3	- 42	- 47	- 51	- 50	- 65	- 91	- 97
1933-4	- 72	- 57	- 66	- 62	- 71	- 86	- 85
1934-5	- 105	- 60	- 73*	- 67	- 83	- 76	- 106

\* Estimated value.

Mean value - 72.0.

Table VIII c. *Mean temperatures (° F.)*

Station ... Year	Long Sutton	Boghall	Newport	Sprow- ston	Wye	Roth- amsted	Seale Hayne
1932-3	39.69	39.18	40.02	39.98	39.26	39.18	44.75
1933-4	38.98	39.47	37.78	38.23	37.66	37.37	39.80
1934-5	44.87	40.18	42.77*	43.52	42.42	42.12	46.89

\* Estimated value.

Mean value 40.67° F.

Table IX. *Analysis of covariance*

Variance due to	D.F.	$[d_t^2]/10^4$	$[T^2]$	$[d_a^2]/10^2$	$[d_t T]$	$[d_t d_a]$	$[T d_a]$
Place differences	6	117.89	40.60	37.79	- 630.99	59.67	- 253.83
Year differences	2	76.91	81.57	11.66	- 791.80	19.13	- 202.72
Residual	10	29.40	16.34	18.49	- 141.43	19.90	- 95.76
Total	18	224.20	138.51	67.94	- 1564.22	98.70	- 552.31

$d_t$  = date of tillering,  $T$  = mean temperature from appearance above ground to tillering,  
 $d_a$  = date of appearance above ground.

From this table, the regression coefficients both of the date of tillering and also of the interval from appearance above ground to tillering can be obtained. The corresponding approximate regression equations are

$$D_T = 227.4 - 3.37T + 0.901d_a,$$

and

$$I = 227.4 - 3.37T - 0.099d_a,$$

respectively, differences between years and places having been ignored.  $D_T$  is the expected date of tillering and  $I$  the expected interval from appearance to tillering.

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The standard errors of the two regression coefficients are in both cases 2.71 and 0.255 respectively, so that neither factor exerts a significant influence, independent of the other, on the interval from appearance to tillering, which is the more interesting of the two dependent variates considered.

The complete analysis of variance for the regression of interval on temperature and appearance above ground is given in Table X.

Table X. *The analysis of regression of interval on mean temperature and appearance above ground*

Variance due to	D.F.	Mean square
Fitting two variables	2	70.10
Residue	8	83.75
Total	10	81.02

The table shows that the combined effect of the two factors is not significant, the variance removed by fitting them being below the residual, although not significantly so. In fact the regression coefficient of the date of tillering on the date of appearance shows that if the temperature were constant at all the stations, a delay of 1 day in the crop's appearance would cause a lag of 0.9 ( $\pm 0.26$ ) days in the time of tillering, this effect being highly significant. Since the partial effect of temperature is not significant, the interval from appearance to tillering is nearly constant. The two factors together remove 72 per cent. of the total variance in the tillering dates, and they account for the significant differences, which, as has already been mentioned, exist in the original data both between the mean tillering dates for each year, and also between those for each station.

Subsequently the mean temperature from the time of sowing to that of appearance above ground was added as an extra variable to the above analyses. The initial of the first tiller is formed during this stage, and hence it was thought that warmer temperatures, increasing the rate of development, might shorten the interval before the tiller actually appeared. This was not found to be the case. Neither the interval nor the date of tillering are significantly influenced by the temperature during this period. Nor does it appear from the data, although a complete analysis was not carried out, that the rainfall between the time of appearance above ground and tillering exerts any influence whatever.

### (2) *Rate of tillering*

As a first hypothesis it is reasonable to suppose that the rate of tillering is influenced by the weather conditions at the actual period of

tillering. The period investigated was that used in determining the rates, that is to say, an interval of about 4 weeks including the tillering date, and the first factor considered was the mean air temperature over this interval. Subsequently the plant number at tillering was included, and the analyses given here as based on these two variables.

It was possible to include the Woburn data in this investigation, the 1933-4 anomaly being irrelevant, and the 1932-3 value being estimated from the whole of the available data. On the other hand, the Long Sutton readings were omitted, the determination of the rates involving many doubtful factors. An alteration in the Rothamsted 1932-3 rate was made, consistent with that already made in the date. This gave a mean tillering rate of 35.7 tillers per hundred plants per week instead of 25.9. At Wye the alteration in date for 1934-5 made no appreciable difference to the rate.

The data finally used are shown in Table XI.

Table XI. *Tillering rates (per hundred plants per week)*

Station ... Year	Roth- amsted	Wye	Sprow- ston	Seale Hayne	Newport	Boghall	Woburn
1932-3	35.7	45.8	44.2	37.1	51.4	55.1	38.2*
1933-4	24.7	44.4	36.2	25.3	45.4	45.2	32.3
1934-5	32.3	15.6	27.8	34.2	38.3*	39.3	22.5

\* Estimated value.

Mean value 36.7.

Table XII shows the mean air temperatures calculated over the respective intervals used in computing the corresponding figures of Table XI, and Table XIII the plant number at tillering at each of the stations.

Table XII. *Mean air temperatures (° F.)*

Station ... Year	Roth- amsted	Wye	Sprow- ston	Seale Hayne	Newport	Boghall	Woburn
1932-3	36.87	45.75	44.07	44.38	45.01	46.52	42.71*
1933-4	42.98	41.67	42.61	39.79	40.70	40.73	41.80
1934-5	41.06	39.05	40.20	48.83	41.54*	38.82	39.09

\* Estimated value.

Mean value 42.10.

The corresponding analysis of covariance is given in full in Table XIV, and shows that there is a significant difference between the mean tillering rates from year to year, but not from place to place.

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Table XIII. *Plant numbers at tillering (per 32 m.)*

Station ... Year	Roth- amsted	Wye	Sprow- ston	Seale Hayne	Newport	Boghall	Woburn
1932-3	1398	1073	1340	1563	480	1548	936*
1933-4	999	1048	971	755	718	1022	910
1934-5	2331	1418	1515	1954	1165*	1845	1119

\* Estimated value.

Mean value 1243.0.

Table XIV. *Analysis of covariance*

Variation	D.F.	$R^2$	$T^2$	$p^2/10^4$	$RT$	$Rp/10^3$	$pT/10^3$
Between places	6	764.22	27.52	141.87	21.31	- 83.37	- 3.72
Between years	2	679.62	24.21	176.05	119.57	- 192.54	- 14.25
Residue	10	510.94	116.31	77.70	129.79	132.88	32.63
Total	18	1954.78	168.04	395.62	270.67	- 143.03	14.66

$R$ =rate of tillering;  $T$ =mean temperature ( $^{\circ}$  F.) over the tillering period;  $p$ =plant number at tillering.

The approximate partial regression equation is obtained from the residual line of the table,  $p$  being expressed in plants per foot:

$$R = -11.14 + 0.7211T + 1.4776p.$$

The first coefficient would occur about 20 times, and the second about 5 times in 100 by chance alone. If it is assumed that, provided an increase in temperature has any effect on the tillering rates, it will increase them, the probability of the first coefficient occurring by chance becomes 0.1, and an association of temperature and tillering rate, independent of the plant number present, is indicated.

The significant positive regression on plant number is of particular interest. Competitive effects, which as subsequent investigations showed are operative at later stages of the crop's development, apparently do not come into action at this early stage. Conditions which are favourable to the production of greater plant numbers are also favourable to the production of more tillers per plant.

The analysis of regression shows that the two factors together remove 44 per cent. of the total variance in the tillering rates. They do not account for the significant differences existing between the mean values for the three years. For this reason some further meteorological factors were investigated, including the rainfall over the tillering period, but none of these appeared to exert any influence on the tillering rates.

A complete set of graphs was also drawn, showing the successive shoot and plant counts for every station in each year, and also the

corresponding weekly rainfall and temperature figures. It appeared possible from these graphs that the occurrence of violent temperature checks between the time of appearance above ground and the time of tillering may exert some influence on the tillering rate. Those crops which suffer the most violent checks appear to tiller most rapidly finally. An even temperature where the growth can continue steadily seems to lead to a low rate of tillering. The effect, if it does in fact exist, is certainly complex, and no numerical expression of it has been arrived at. The point is suggested here merely as a possibility which may be worth investigating when further data are available.

## VI. MAXIMUM SHOOT NUMBER

The curves given in Section II show that in general the number of shoots per unit length reaches a maximum in early spring. The mean number of shoots is very different in each of the three years, and it was thought that the differences might be associated with differences in the mean temperature either from the time of sowing, or from appearance above ground, to the time when this maximum is reached. The analyses of maximum shoot number and mean temperature in the latter period did not show this to be the case, for no significant regression appeared. The connection with rate of tillering and plant number at tillering was then investigated. The rates of tillering and the plant numbers at tillering which are required are included in those shown in Tables XI and XIII respectively, with the exception of the Plumpton values. The 1932 Plumpton rate was estimated as 39.7 and the plant numbers as 769, while the 1933-4, 1934-5 values are 38.4, 21.4 tillers per hundred plants per week, and 797, 844 plants respectively. Table XV shows the corresponding maximum shoot numbers.

Table XV. *Maximum shoot numbers (per 32 m.)*

Station ... Year	Seale Hayne	Roth- amsted	Newport	Boghall	Sprow- ston	Plumpton
1932-3	4836	5017	2680	5238	3670	3122*
1933-4	3715	2899	2998	3490	3213	2678
1934-5	4291	8133	5875	5967	4995	4106

\* Estimated value.

Mean value 4273.

The analysis of covariance is given in Table XVI, the tillering rates having been expressed per 1000 plants per week for convenience.



Table XVI. *Analysis of covariance for maximum shoot number (per 32 m.)*

Variance due to	D.F.	$[s^2]/10^3$	$[R^2]$	$[p^2]/10^3$	$[sR]/10$	$[sp]/10^3$	$[Rp]/10$
Places	5	83058	69327	18790	81	33783	- 8822
Years	2	175100	42455	15414	- 36887	51897	- 9870
Residue	9	92003	26915	8150	11801	13750	12473
Total	16	350161	138697	42354	- 25005	99430	- 6219

$R$  = tillering rate per 1000 plants per week;  $p$  = plant number per 32 m.;  $s$  = maximum shoot number per 32 m.

The partial regression coefficients of maximum shoot number on plant number and tillering rate, as computed from the residual line of this table, are:

$$b_p = 3.495 \pm 1.868,$$

$$b_R = -11.810 \pm 10.280,$$

respectively. The first regression coefficient, although not fully significant ( $P \approx 0.1$ ), indicates a probable association between the maximum shoot number and the plant number at tillering. If this is a true association, the maximum shoot number is greater by three or four shoots per unit length for each additional plant per unit length present at tillering. The rate of tillering does not appear to exert any discernible influence independent of that exercised by the plant number.

The analysis of regression shows that the two factors together do not remove a significant fraction of the total variance, nor do they account for the significant differences which exist between the mean maximum shoot numbers for the three years, in the original data.

Other factors associated with the maximum shoot number have not been found. It is a measurement which appears to possess less interest than might perhaps be anticipated, since subsequent investigations have shown that there is little association between it and the final yield.

## VII. MAXIMUM GROWTH RATE

The period of maximum growth rate is defined as that at which the shoot height is increasing most rapidly, and, in general, occurs in late May or early June. At the end of this period the growth rate diminishes rapidly and the ears emerge. The mean shoot-height curves obtained from four stations in each of the three available years have already been shown in Fig. 1. Both the 1932-3 and the 1933-4 curves show the diminution in growth rate at the end of the period. In 1934-5 the readings

were not continued long enough at a sufficient number of these stations to allow this stage to be well shown.

The maximum growth rates can be estimated by fitting cubic curves to the corresponding observational curves for the individual stations. Five observations are sufficient to allow reliable estimates of all the constants of the cubic to be made, provided one or two of these have been taken after the growth rate begins to diminish. The steepest gradient of the cubic curve gives the growth rate required. If the end-readings have not been taken this rate is still determinable, although the date on which it occurs cannot be well estimated.

Largely on account of insufficient observations, several stations had to be omitted from the maximum growth rate investigations. These stations were:

(1) Wye, where sufficient readings were not taken in 1932-3 and the crop suffered badly from Take-All in 1934-5.

(2) and (3) Seale Hayne and Woburn where sufficient readings were not taken in either 1933-4 or 1934-5.

(4) Long Sutton, where the crop failed entirely in 1933-4, and in 1934-5 was very patchy and poor by the time of maximum growth rate.

The remaining five stations therefore provided all the available information. Table XVII shows the mean rate for the two varieties at each of these stations, measured in centimetres per day.

Table XVII. *Maximum growth rates in cm./day*

Station ... Year	Rothamsted	Newport	Boghall	Sprowston	Plumpton
1932-3	1.75	2.25	2.15	2.24	2.16*
1933-4	2.10	2.83	2.13	2.71	1.87
1934-5	2.33	2.42	1.94	1.79	2.82

\* Estimated value.

Mean value 2.23.

It appeared likely that these maximum growth rates would be largely influenced by the weather conditions prevailing about the time of rapid growth. The period chosen for investigation was an interval of about 5 weeks, from about the middle of May to the end of June, varying for each station, during which the individual graphs showed the respective shoot heights to be increasing rapidly. It was substantially the same period as that used in determining the maximum growth rates.

Since the growth rate considered is a rate of elongation and the elongation of a plant takes place largely at night, it seemed advisable to con-

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sider both the mean maximum and the mean minimum temperature over this period, rather than just the mean temperature; for the minimum temperature gives a better indication of the average night temperature than does the general mean.

The shoot height at ear emergence was also included in the analyses, for it had already appeared that this measurement is highly correlated with the yield of grain, and it was probable that it would also be correlated with the growth rate, since the more rapid is the latter, the greater the shoot height at ear emergence is likely to be. Owing to these inter-correlations, if the period of rapid growth were a critical one, especially in respect of temperature, so far as the yield were concerned, then the correct interpretation of a straight regression of maximum growth rate on temperature would be uncertain.

Preliminary investigations showed that each of the straight correlations existing between the mean maximum temperature, and the quantities, maximum growth rate, minimum temperature, and shoot height at ear emergence, would occur at least 20 times in 100 by chance alone. The correlation with the maximum growth rate would occur between 50 and 60 times in two sets of random numbers. On these grounds the mean maximum temperature was omitted from the subsequent analysis of covariance.

The possibility of the maximum shoot number exerting some influence on the maximum growth rate was also investigated, but the straight correlation existing between the two factors is only  $-0.33$ , which is well below the  $0.1$  level of significance. In addition, the straight correlation between maximum shoot number and shoot height at ear emergence is quite insignificant, so that there was no probability that the effect of maximum shoot number was being masked by the shoot-height effect. The maximum shoot number was therefore not considered further.

Table XVIII. *Mean daily minimum temperature ( $^{\circ}$  F.)*

Station ...	Rothamsted	Newport	Boghall	Spröwston	Plumpton
Year					
1932-3	46.60	45.95	46.18	45.19	48.51*
1933-4	46.28	44.83	45.33	49.08	45.34
1934-5	44.91	43.23	42.89	43.91	49.84

\* Estimated value.

Mean value  $45.87^{\circ}$  F.

The mean minimum temperatures and the shoot heights at ear emergence are shown in Tables XVIII and XIX, and the analysis of covariance in Table XX.

Table XIX. *Shoot heights at ear emergence (cm.)*

Station ... Year	Rothamsted	Newport	Boghall	Sprowston	Plumpton
1932-3	64.4	78.2	83.7	72.3	71.0*
1933-4	71.8	89.6	82.4	65.9	61.5
1934-5	87.3	82.6	69.9	60.2	83.6

\* Estimated value.

Mean value 75.0 cm.

Table XX. *The analysis of covariance*

Variance due to	D.F.	$[g^2] \times 10^2$	$[h^2]$	$[T_{\min}]^2$	$[gh] \times 10$	$[gT_{\min.}] \times 10$	$[hT_{\min.}]$
Places	4	38.83	522.33	20.20	44.47	1.66	-65.90
Years	2	12.44	23.28	6.53	5.36	3.59	-12.28
Residue	7	114.82	750.81	29.84	243.66	45.39	68.24
Total	13	166.09	1296.42	56.57	293.49	40.14	-9.94

$g$  = maximum growth rate (cm./day);  $h$  = shoot height at ear emergence (cm.);  $T_{\min}$  = mean minimum temperature during interval considered ( $^{\circ}$  F.).

It appears from the last table that neither the differences in maximum growth rate from year to year, nor from place to place, are significant. In fact both these variations are below the residual variation, although not significantly so.

The approximate regression equation, computed from the residual line and giving the maximum growth rate to be expected at any station in any year, is

$$G = -4.04 + 0.0235h + 0.0983T_{\min.}$$

The standard errors of the two partial regression coefficients are  $\pm 0.0066$  and  $\pm 0.0330$  respectively. Hence, in addition to the anticipated significant correlation existing between the maximum growth rate and the shoot height at ear emergence, the minimum temperature exerts a significant influence on the maximum growth rate, which is independent of the shoot height attained. It should be noticed that these results are based on a residual variance corresponding to five degrees of freedom only, and therefore need to be confirmed when more data are available from the subsequent years of the scheme.

The analysis of variance is given in Table XXI, which shows that 84 per cent. of the total variance is removed by fitting the two variables. Such a reduction would occur by chance alone less than once in 100 times.

Table XXI. *The analysis of variance*

Variance due to	D.F.	Mean square
Fitting 2 variables	2	0.5597
Residue	5	0.0258
Total	7	0.1640

## VIII. THE YIELD OF GRAIN PER ACRE

An investigation was made into the influence of weather conditions prevailing during the harvest year on the yield of grain per acre. When further years of the scheme have elapsed and more data is available, it will be possible to classify the stations in groups, according as they possess light, medium or heavy soils, but at present this is impossible, owing to the scantiness of the data, and only the broadest effects could be looked for. No results, in which any degree of confidence could be placed, were obtained. Four variables were considered, namely the winter and summer rainfall and the winter and summer temperature. Each of these is a weighted mean over about 18 weeks, the weighting being heaviest over the two months December and January, in the case of the winter variables, and over May and June in the case of the summer variables. The matrix of straight correlations, from which year and place effects have been eliminated, is shown in Table XXII. The only connection which approaches significance is that with the summer rainfall,

Table XXII. *Residual correlations existing between yield and meteorological factors*

	$y$	$R_1$	$R_2$	$T_1$	$T_2$
$y$	—	0.0235	0.5525	0.1491	-0.0356
$R_1$	—	—	0.4077	0.2391	0.5556
$R_2$	—	—	—	0.7297	-0.0360
$T_1$	—	—	—	—	0.0052

$$r_{(P=0.05)} = 0.5760 \quad (n=10).$$

which would occur 8 times out of 100 by chance alone. Further investigation of this effect leads to the apparent conclusion that the yield increases by about 1.8 cwt. per acre for each additional inch of rain in May and June. It is considered, however, that this is in all probability a chance effect which is not likely to appear when more data are available. It does not agree with the results obtained from the study of the Broadbalk wheat plots at Rothamsted which all show a decrease in the yield varying from 0.3 to 0.8 cwt. per acre for an additional inch of rain at this time of year. In addition the analyses show that the place effect is not in agreement with this residual result, places with the highest yields having the driest weather in May and June. This, of course, may be due to the action of other factors, but it agrees with the conclusion that the association obtained above is purely fortuitous—a conclusion which is strengthened yet again by the high correlations appearing between certain

of the weather factors at different times of the year, such as that between winter temperature and the succeeding summer rainfall, which can only be regarded as a chance association. It appears that nothing is to be gained by a more detailed investigation until further data have accumulated.

### IX. SUMMARY

The paper gives an account of the results obtained from the data provided by the first three years of the Sampling Observations on Wheat of the Crop-Weather Scheme.

Section I describes the Sampling Observations on Wheat of the Crop-Weather Scheme which have been initiated in order that the effect of weather conditions may be studied at all stages of the wheat crop's growth from germination to maturity.

In Section II several curves are given which illustrate the progress of the wheat crop. The shoot-number curves bear a marked resemblance to one another subsequent to the period when the shoot number is a maximum in spite of the wide divergences which exist between the maximum shoot numbers themselves.

Section III indicates the statistical processes involved in the analyses given in the succeeding sections.

Sections IV-VIII are devoted primarily to discussions of the effects of various meteorological factors on specific stages of the crop's growth. The following results have emerged:

The length of the interval from sowing to appearance above ground is shown to be largely dependent on the mean soil temperature during this interval, the relation being well expressed by a quadratic regression. The growth rates of the plants at this time are shown to be in good agreement with those which would be obtained by applying Van 't Hoff's law. Neither the rainfall nor the variation in temperature during the period appear to affect its length.

Squarehead's Master appears above ground consistently later than Yeoman, but the amount of this lag is apparently uninfluenced by variations in soil temperature.

The tillering date is substantially determined by the date of appearance above ground, a lag of one day in the date of appearance causing a lag of 0.9 ( $\pm 0.26$ ) days in the date of tillering. The length of the interval from appearance to tillering is therefore practically constant, the mean temperature during the interval exerting no discernible influence.

The rate of tillering increases significantly with the plant number at

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tillering, and shows indications of an association with the temperature at the time of tillering. The two factors do not account for the significant differences which exist between the mean values of the rates for the three years.

The maximum shoot number shows evidence of an association with the plant number at tillering, but appears to be uninfluenced either by the rate of tillering, or by the mean temperature during the interval from appearance above ground to the time when the shoot number is a maximum.

The maximum growth rate is significantly influenced by the mean minimum daily temperature during the period of rapid shoot growth, independently of the height which the shoots attain at the end of this period. As is to be expected a significant correlation exists between this height and the maximum growth rate.

Neither the maximum temperature at this time, nor the maximum shoot number appear to exert any influence on the maximum growth rate.

The yield of grain per acre cannot be said to show definite association with any of the four weather factors considered, namely, mean summer and mean winter temperature and mean summer and mean winter rainfall. Until further data have accumulated, so that the stations can be divided into classes according to the types of soil they possess, there appears to be little point in carrying out a more detailed investigation on this point. It does appear, however, that over the range of weather conditions prevalent in this country the wheat crop tends to compensate itself for any checks it may receive from temporarily adverse weather conditions. For this reason it is doubtful whether reliable predictions of yield will be obtained from a consideration of the weather conditions alone.

The relations existing between the yield and prior measures of crop's growth have also been examined but are not discussed in detail in this paper. It is intended to publish a full account of this aspect of the results when the 1935-6 observations are available. A brief account of the relations found to hold during the three years under discussion has been published in the *Journal of the Ministry of Agriculture* (3). It was found that the yield of grain was very closely connected with the shoot height at ear emergence, and a negative correlation with plant number was also observable. The differences between places were well accounted for by the resultant regression formula, and residual discrepancies between years can be fairly attributed to extraneous causes.

I am very glad to thank Mr F. Yates, Chief Statistician, Rothamsted Experimental Station, for the assistance and advice he has given me throughout the course of this investigation.

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# A NOTE ON THE ANALYSIS OF COVARIANCE

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1. In recent papers which refer to the use of covariance there has still appeared an occasional lack of understanding of this method of statistical analysis. There have, for example, been references in the literature to methods of analysis which the writer has used in certain problems as if they were in competition with the use of covariance as described by Fisher (1, 2). It would therefore seem to be of value to indicate briefly the different situations in which covariance may prove of use, in order that the distinction between some of these situations may be clearly realised.

Covariance is widely used in order to see how far one variable can be expressed in terms of another, that is, in statistical terminology, in the calculation of regression coefficients. In studies of the simultaneous variation of more than one biological quantity, other methods involving the use of covariance, such as, for example, a survey of correlations, will of course sometimes be appropriate; only, however, the regression problem is considered here. This problem can in its turn be broadly divided into two distinct types, one in which regression is used for observational data of a general kind, the second for data arising from a controlled experiment, in which, to improve our accuracy, our final figures are to be adjusted by means of an initial set of data. One object of the former type of analysis, of data in general, will be to detect any sources of heterogeneity, and since heterogeneity can arise in various ways, all of these may have to be looked into. Thus if the data are classified in groups, heterogeneity in the regression coefficients for the different groups might be one of these ways. An example of a complete analysis of data of this kind was given by the writer in (3). In an experiment, however, the analysis is simplified, for our first object is to see whether the *treatments* have introduced heterogeneity. Without covariance, this is of course done in the usual way by comparing the treatments' variance with the error variance, for if the treatments have had no effect, and the experiment has been properly randomised, we expect

these to be the same. If we are able to reduce our error by correlation with an "initial" set of figures which cannot have been affected by the treatments, we compare this adjusted error variance with an appropriate adjusted treatments' variance. The exact  $z$  test of significance to be used for this purpose has been given by Fisher<sup>(1)</sup> and by the writer<sup>(4)</sup>. The treatments' (or subgroup of treatments) sum of squares so obtained is not in this test split up any further, the test given being the logical one for the object stated.

In any summary of adjusted treatment means, it is usually sufficient to obtain a standard error in the ordinary way from the adjusted error variance. This will always tend to underestimate the true significant difference between two treatments, but as the discrepancy will be slight will, in conjunction with the exact  $z$  test of the treatments (or subgroup of treatments) as a whole, normally be all that is required. The calculation of the exact standard error for any treatment difference would, on the other hand, be advisable for the adjustment by non-random "initial" differences described below (see, for example, (7), p. 54).

It may be noticed that if the error regression coefficient differed somewhat in different blocks, a slightly more efficient analysis might be made by taking out a separate regression coefficient for each block. These coefficients cannot, however, easily be separated from the treatment means, and for properly randomised experiments, in which the usual analysis is always a valid one, this modification would hardly ever be worth while. In a discussion<sup>(5)</sup> on this point in the design and analysis of dairy cow nutrition experiments, this conclusion was made provisionally, but has been substantiated by the actual results of an experiment conducted and analysed on the lines proposed<sup>(6)</sup>.

For the benefit of those doubtful of the correct procedure for missing "plots" in covariance problems, it is recalled that in the nutrition experiment referred to, the yield for a "missing cow" had to be estimated, and it was pointed out that *both* the initial and final yields should be estimated by the usual formula (even if the true initial yield is available).

2. Covariance may be used with experimental data, not for the purpose of correcting final yields, but for examining the dependence of successive or concurrent sets of observations on each other, even if it is known that our treatments may have affected both sets of data (see, for example, (8)). Although the data tend then to be of a type comparable with observational data in general, so that methods of analysis, and the interpretation put on them, depend more on the particular data in

question, the conclusions that may be drawn from different statistical results can, to some extent, be indicated. It might, for example, be possible to show in a wheat experiment that treatments have affected the numbers of wheat plants, and also the numbers of tillers counted later, but that differences in the numbers of tillers appear to be entirely or largely accounted for by the differences in plant numbers. Conversely, differences in plant numbers might not reach significance, nor differences in tiller counts, but owing to a smaller error variance, the tiller counts when adjusted for plant numbers might enable us to establish significant treatment effects. Strictly speaking, this latter case still corresponds to the use of covariance for improving accuracy, provided its meaning is understood. Similarly, when no preliminary figures have been taken, we are sometimes obliged in analysing experiments with perennial crops, especially with those from mature trees, to use the first year's experimental records as a pseudo-uniformity trial, provided the treatments have not had time to influence very appreciably this first year's records.

A third possibility in our wheat experiment is the existence of significant plant and tiller count differences, but still significant tiller count differences after adjustment for plant numbers; the interpretation we then put on these adjusted tiller counts must necessarily be less definite, as we cannot be certain how the treatments would have affected the numbers of tillers if the plant numbers had been different from what in fact they actually were. Thus in an experiment at Long Wittenham in 1934 to obtain information on the disposal of straw under mechanised farming conditions, plant counts made in February by sampling showed a significant depression in numbers with "straw ploughed in with sulphate of ammonia", in comparison with "no straw", but tiller counts in July a significant increase. Tillers adjusted for plant numbers gave a much smaller standard error, and stressed of course the much greater proportionate gain in tillers with the "straw ploughed in" treatment, but these adjusted figures tend here to be somewhat incidental; the increased tillering will, for example, depend to an unknown extent on any interaction between the depression in plant numbers and the subsequent effect of the treatment.

The conclusions that may rigorously be drawn from certain types of non-randomised experiments are of a similar kind. Thus it may be possible to show, if there are systematic initial differences between experimental units, that apparent final differences disappear after adjustment, so that there is no real evidence for treatment effects; but the alternative conclusion, that if apparent treatment differences still remain, the effect of

treatments must be established, is clearly invalid. If in a trial on the possible advantage of milk to children, where adequate randomisation was not undertaken, no evidence was found of initial differences between the groups of children used, it could be argued that any evidence for subsequent observed differences being due to the treatments was somewhat strengthened; but the same limitations in experimental design will be present, and the possibility of subsequent group differences arising from non-treatment causes must still be considered, as experience with this kind of trial has shown.

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# SERUM PHOSPHATASE IN THE DOMESTIC FOWL<sup>1</sup>

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(With Four Text-figures)

AUCHINACHIE AND EMSLIE (2) have recently reviewed the significance of phosphatase estimations in the domestic fowl; their paper also describes their own extensive observations and experiments on the serum phosphatase activity of the fowl under different conditions. The present communication deals with somewhat similar experiments which were undertaken with the object of throwing additional light on the metabolism of calcium and phosphorus in the laying fowl.

Mineral metabolism experiments have suggested that the skeletal metabolism of the fowl may be affected by demands for calcium for shell formation when the calcium intake is unable to provide for sustained shell production (9, 10). The laying period may also be preceded by a short period (2-3 weeks) of increased calcium and phosphorus retention. Since it is generally held that serum phosphatase activity is affected by interference with the normal metabolism of calcium and phosphorus (1, 2) as well as by various bone disorders (13), it appeared possible that laying might be associated in the fowl with changes in serum phosphatase activity. The present experiments were carried out in order to test further these hypotheses.

## ANALYTICAL METHODS

Blood samples were drawn from the wing veins in the case of adult birds, and from the external jugular in the case of younger chickens. The samples were always drawn in the afternoon between 4 and 6 p.m. They were allowed to clot spontaneously, and were placed in the refrigerator within an hour of sampling. The serum was separated by centrifugation, and the analyses were carried out on the same evening.

Serum phosphatase and inorganic phosphorus estimations were made in accordance with the instructions of Bodansky (3), (4), and serum phos-

<sup>1</sup> Part of a thesis presented to the University of London for the Degree of Doctor of Philosophy.

phatase activity is reported in Bodansky units. Serum calcium was determined by the Clark-Collip modification of Tisdall's method (8). This method is not suitable for accurate work with the sera of laying birds, but its rapidity and convenience commended its use as a clinical check.

"Tibia ash" was determined by extracting the tibio-tarsus plus fibula for at least 24 hours in several changes of hot 95 per cent. alcohol, and then completing the extraction with ether in a Soxhlet apparatus. The fat-free bones were dried to constant weight in an electric oven at 100° C., and then ashed to whiteness in an electric muffle at about 700° C.

#### PRELIMINARY OBSERVATIONS

Since no data were available on the blood phosphatase of poultry when the observations began, it was considered advisable to conduct a preliminary survey. For this purpose analyses were made on blood samples drawn from representative adult birds in a laying flock of White Wyandottes kept under semi-intensive management and feeding. Egg production has been high and mortality low in this flock over a number of years.<sup>1</sup>

The data secured in this preliminary survey are summarised in Tables I-IV. Some of the data have been reported previously (11).

Table I. *Laying hens (thirty-two birds)*

	Serum phosphatase Bodansky units	Serum inorg. P mg./100 ml.	Serum Ca mg./100 ml.
Average value	17.1	5.2	25.3
Highest value	40.2	7.1	31.8
Lowest value	3.6	3.0	16.4

The thirty-two values for serum phosphatase were distributed as follows:

Serum phosphatase range (Bodansky units)	0-4	4-8	8-12	12-16	16-20	20-24	24-28	28-32	32-36	36-40	40-44
No. of determinations	1	3	10	4	4	2	2	4	0	1	1

No correlation could be traced between serum phosphatase and intensity of egg production as measured by the trap-nest records for the 4 weeks before sampling. Auchinachie and Emslie also failed to trace any direct connection between serum phosphatase and number of eggs laid.

<sup>1</sup> The flock in question is the field flock at the Poultry Research Institute, Hillsborough, Co. Down.

The values obtained for cocks from the same flock are summarised in Table II.

Table II. *Cocks (five birds)*

	Serum phosphatase Bodansky units	Serum inorg. P mg./100 ml.	Serum Ca mg./100 ml.
Average value	4.7	4.8	9.8*
Highest value	6.8	5.2	—
Lowest value	3.0	3.9	—

\* Only one determination made.

Four adult cocks from another source gave similar values with the exception of one bird which gave a value of 15.1 units.

Data were also secured for birds from the same flock which were not in full lay at the time of sampling. These figures are summarised in Table III, but it should be borne in mind that some of these birds had only stopped laying a short time previously, while others were coming into lay again. Furthermore, it is a debatable point whether a bird from a heavily laying strain kept under intensive or semi-intensive conditions is ever in a true "non-laying" condition (*i.e.* with the reproductive system completely inactive) once she has entered upon her productive career, and all the birds in the flock had previously laid well. The values summarised in Table III are to be regarded as values for non-laying birds, subject to these qualifications.

Table III. *Adult hens not in lay (twelve birds)*

	Serum phosphatase Bodansky units	Serum inorg. P mg./100 ml.	Serum Ca mg./100 ml.
Average value	13.1	5.4	23.7
Highest value	19.3	7.9	26.0
Lowest value	8.0	4.1	15.0

It will be noted that the serum calcium values approach those for laying birds; this also suggests that the birds were not in a true non-laying condition.

Blood samples were drawn from a number of the birds on two occasions, usually at an interval of about 3 months. The data for sixteen of these birds, which were in full lay on both occasions of sampling, are given in Table IV.

The figures in Table IV display the same wide variation from bird to bird as the other figures for laying birds. It is remarkable that, with only two exceptions, individual laying birds tended to have serum phosphatase and inorganic phosphorus values of the same order about 3 months after the date of the first sampling.

Table IV. *Laying birds sampled twice (sixteen birds)*

No. of bird	Date of sampling	Serum phosphatase units	Serum inorg. P mg./100 ml.	Serum Ca mg./100 ml.
3100	8. ii. 34	9.2	6.7	—
	21. iv. 34	6.8	6.4	29.0
3628	8. ii. 34	7.5	5.6	—
	21. iv. 34	12.7	2.8	25.6
3043	21. iv. 34	11.9	5.8	22.0
	25. iv. 34	9.6	5.3	22.6
3605	5. iv. 34	39.6	6.1	24.0
	25. iv. 34	9.9	5.1	25.0
3601	17. ii. 34	8.3	5.6	—
	25. iv. 34	10.8	5.2	21.0
3063	27. ii. 34	16.4	4.8	19.7
	2. v. 34	16.9	3.9	24.6
3617	27. ii. 34	9.2	6.4	23.1
	2. v. 34	14.5	4.8	26.6
3623	12. iii. 34	15.4	4.0	23.5
	28. vi. 34	8.4	4.8	—
3620	12. iii. 34	16.7	3.3	19.8
	7. vi. 34	21.8	4.3	23.4
3071	12. iii. 34	27.7	4.1	23.6
	7. vi. 34	32.8	4.2	27.0
3047	20. iii. 34	11.7	6.8	36.2
	28. vi. 34	8.1	6.1	—
3076	20. iii. 34	14.0	6.8	30.0
	28. vi. 34	15.4	7.1	27.5
3607	20. iii. 34	17.1	6.0	26.1
	7. vi. 34	14.0	4.7	29.0
3629	12. iii. 34	40.2	5.5	33.2
	7. vi. 34	14.0	6.3	12.8
3059	27. iii. 34	12.2	4.9	20.0
	7. vi. 34	10.6	5.0	26.8
3062	27. iii. 34	29.2	4.8	24.2
	28. vi. 34	28.4	5.8	20.9

Some determinations on pullets, which were almost mature but had never laid, gave the following values:

Table V. *Adult pullets, ovaries quiescent (nine birds)*

	Serum phosphatase units	Serum inorg. P mg./100 ml.
Average value	3.5	4.5
Highest value	5.3	5.9
Lowest value	1.7	3.6

Another pullet in this group of birds gave a serum phosphatase value of 12.1. An egg weighing 4.4 gm. was found in the ovary, which was showing signs of activity. The values for these pullets are lower than those for laying birds and are of the same order as the values for cocks;



however, since these pullets came from another source, they were not strictly comparable with the birds in Tables I-IV.

The preliminary work which has been reported above prompted the following tentative considerations in regard to serum phosphatase in the fowl:

(a) The values for laying White Wyandotte hens were higher than those for the cocks in the same flock. The values for laying hens showed a considerable degree of variation for bird to bird.

(b) The values for ten pullets which had never laid were of the same order as the values for cocks, but these pullets were from another source and therefore not strictly comparable.

(c) No direct connection was observed between serum phosphatase and intensity of egg production.

(d) Adult hens which had laid well but were not laying at the time of sampling gave values not very different from the values for hens in full lay.

(e) Individual laying hens usually had serum phosphatase values of the same order 3 months after the first determination.

At this stage the preliminary observations were brought to a close, and experiments were commenced under more closely controlled conditions.

#### EXPERIMENT I

This experiment was carried out in order to trace the serum phosphatase activity of normal birds from the day of hatching until maturity.

A batch of chicks from a pedigree White Wyandotte flock was hatched on May 17, 1934. A number of the day-old chicks were removed and bled for the appropriate analyses. The remaining chicks were reared on the following ration:

Bran	...	...	...	...	20 parts.
Sharps	...	...	...	...	10 parts.
Sussex ground oats	...	...	...	...	10 parts.
Maize meal	...	...	...	...	10 parts.

Fortified cod-liver oil: 4 lb. per cwt. meal mixture.

Scratch grain: From hatching until 5 weeks, 2 parts pinhead oatmeal, 1 part cracked yellow maize. From 5 weeks onward, whole wheat.

Separated milk only to drink *ad libitum*.

High-grade limestone grit *ad libitum*.

This rearing ration has always given very good results, and has proved most dependable in practice.

The chicks were allowed out on grass runs from the third week onwards, except in bad weather. Their health and condition remained good

throughout the experiment. At suitable intervals birds were removed and bled for the blood analyses.

When the birds came into lay (November, 1934), the rearing ration was replaced by the following ration, which gives very good results with birds on range:

Bran	...	...	...	...	...	2 parts.
Pollards	...	...	...	...	...	4 parts.
Maize meal	...	...	...	...	...	2 parts.
Sussex ground oats	...	...	...	...	...	1 part.
Extracted soya-bean meal	...	...	...	...	...	1 part.
Salt	...	...	...	...	...	0.5 %.
Oyster-shell grit	<i>ad libitum</i> .					
Water to drink.						

Scratch grain: equal parts of wheat, cracked yellow maize and oats.

### *Experimental results*

The average data for male birds are set out in Table VI and those for female birds in Table VII.

The same figures appear for both male and female birds up to the age of 20 days; this is because pooled samples from birds of both sexes were used until the birds were large enough to yield sufficient blood for the analyses from one bird. Since no differences between the sexes in respect to serum phosphatase were apparent from 26 days of age until the onset of laying, it did not appear likely that there were any large differences between the sexes in this respect before 26 days of age.

The live-weight figures indicate satisfactory growth, although the strain of White Wyandottes used in this work has sometimes shown itself capable of more rapid growth.

The serum calcium values are normal, except for some high values at 6 and 7 weeks of age. The values for pullets show the large increases normally associated with the onset of laying (7, 14, 15).

The serum inorganic phosphorus values are normal. The gradual fall towards a lower level from the sixteenth week onwards was also noted by Harshaw, Fritz and Titus<sup>(12)</sup> in their work with normal Rhode Island Reds.

The values for tibia ash accord on the whole with those found by Harshaw, Fritz and Titus for normal Rhode Island Reds.

The most striking features of the results relate to the serum phosphatase values. A high initial value of 80-90 Bodansky units was followed by an increase to a maximum value of about 110 units during the second week. During the third and part of the fourth week the value decreased again sharply to a value of 40-50 units. The values for male

Table VI. *Average data; male birds*

Age days	No. of birds	No. of samples	Live weight gm.	Tibia ash %	Serum Ca mg./100 ml.	Serum inorg. P mg./100 ml.	Serum phosphatase Bodansky units
1	40	5	39.8	32.7	11.2	7.2	88.7
8	10	3	52.6	36.3	11.8	7.9	106.2
12	10	2	73.6	34.4	11.6	7.1	114.2
16	12	4	96.6	41.6	11.5	7.8	85.6
20	10	5	128.2	42.1	12.7	7.2	67.5
26	2	2	144.3	45.4	12.4	8.6	42.7
33	2	2	293.8	49.5	11.2	8.3	44.9
40	1	1	375	44.6	12.2	8.1	43.6
47	3	3	389	45.3	12.3	6.6	39.0
61	4	4	680	44.0	10.2	7.0	35.1
75	3	3	1039	44.0	11.8	7.5	40.0
89	3	3	1393	—	11.5	6.5	32.5
126	3	3	2133	—	10.8	6.0	26.2
151	3	3	2858	—	9.6	5.8	23.9
200	2	2	3363	—	9.8	4.3	14.8
235	2	2	—	—	10.7	4.0	10.8
265	2	2	—	—	12.3	2.5	9.2
302	2	2	3213	—	11.4	3.4	9.5
329	2	2	—	—	—	3.0	6.5

Table VII. *Average data; female birds*

Age days	No. of birds	No. of samples	Live weight gm.	Tibia ash %	Serum Ca mg./100 ml.	Serum inorg. P mg./100 ml.	Serum phosphatase Bodansky units
1	40	5	39.8	32.7	11.2	7.2	88.7
8	10	3	52.6	36.3	11.8	7.9	106.2
12	10	2	73.6	34.4	11.6	7.1	114.2
16	12	4	96.6	41.6	11.5	7.8	85.6
20	10	5	128.2	42.1	12.7	7.2	67.5
26	3	3	132.7	42.8	10.4	7.9	49.5
33	4	4	214.8	44.0	11.6	8.7	43.4
40	3	3	342	45.4	12.7	8.6	43.9
47	2	2	374	45.5	13.0	7.7	36.2
61	2	2	655	46.0	10.7	7.7	30.7
75	3	3	807	—	10.0	7.0	35.3
89	3	3	1123	—	11.6	7.4	35.8
126	4	4	1563	56.6	11.1	6.0	22.1
151	3	3	2025	—	11.4	5.4	23.6
200	5	5	2510	—	20.4	4.0	17.3
207	3	3	2450	—	22.0	3.5	45.4
235	6	6	—	—	19.8	3.3	47.5
265	3	3	—	—	22.7	3.6	54.8
267	3	3	—	—	20.9	2.3	18.3
299	3	3	2483	—	19.8	2.8	41.4
300	3	3	2525	—	24.2	4.1	23.4
327	6	6	—	—	21.9	3.9	43.0

birds continued to decrease fairly uniformly from about 45 units at 4 weeks of age to about 5 units at 46 weeks. The value of 5 units is probably not far from the normal value for adult cocks.

The values for the female birds followed the same course as the values

for the male birds until the onset of laying at about 30 weeks of age; when laying began the values increased, but to a very different degree in different birds, although individual variations had been relatively small previously. Average values for pullets after the beginning of laying are included in Table VII, but it must be remembered that they are the averages of widely differing values. The low values for pullets before laying confirm the preliminary observations on pullets.

It is not easy to ascribe the relatively sudden increases in serum phosphatase at the beginning of laying to vitamin-D deficiency. The birds received a ration which would ordinarily be regarded as adequate in this factor for birds on open grass range, and egg production was very satisfactory. Birds under satisfactory conditions have the capacity to store sufficient vitamin D to meet some weeks at least of egg production (6).

## EXPERIMENT II

This experiment was carried out in order to see if the increases in serum phosphatase with the onset of laying could be reduced, or if their range of variation could be narrowed, by the administration of vitamin D.

After the birds in Exp. I began to lay, they were divided into two groups of six pullets and two cockerels each. One group was continued under the same conditions as before. The other group was placed under identical conditions but the birds received  $\frac{1}{2}$  ml., increased after a short time to 1 ml., of radiostol B.D.H. (3000 international units vitamin D per ml.) per bird per diem. The radiostol was given orally, commencing on December 11, 1934. Even if allowance be made for the relatively poor vitamin activity of irradiated sterol preparations as compared with the vitamin D of cod-liver oil for chickens (18), 3000 international units per diem appeared sufficient to meet all requirements for egg production (6, 17).

The data from this experiment are set out in full in Table VIII. It does not appear from these results that the radiostol exercised any appreciable effect on the serum phosphatase values and wide individual variations occurred. This confirms the experience of Auchinachie and Emslie in the case of similar but more extensive experiments. It is noteworthy that individual pullets tended in general to display serum phosphatase values of the same order throughout the experiment. This is in agreement with a similar observation made during the preliminary work. No explanation is offered for the very high values obtained in the cases of pullets 4701 and 4713.

Table VIII

Radiostol group										No Radiostol group					
Pullet No.	Date	Eggs laid to date	Live weight kg.	Serum phosphate unites	Serum inorg. P mg./100 ml.	Serum Ca mg./100 ml.	Date of first egg	Pullet No.	Date	Eggs laid to date	Live weight kg.	Serum phosphate unites	Serum inorg. P mg./100 ml.	Serum Ca mg./100 ml.	Date of first egg
4709	3. xii. 34	22	2.55	19.4	2.8	21.0	4. xi. 34	4706	3. xii. 34	0	2.75	21.3	6.1	25.0	4. xii. 34
	7. i. 35	50	—	19.9	4.3	19.8	—	—	8. i. 35	27	—	63.1	3.5	23.0	—
	6. ii. 35	72	—	29.6	4.2	20.2	—	—	6. ii. 35	50	—	18.7	3.6	23.8	—
	12. iii. 35	99	2.53	22.9	3.7	24.8	—	—	13. iii. 35	78	2.73	19.9	5.6	27.8	—
	8. iv. 35	120	2.43	62.5	3.2	23.0	—	—	9. iv. 35	98	2.85	21.4	4.8	32.0	—
4701	10. xii. 34	18	2.83	89.7	3.5	21.8	14. xi. 34	4704	10. xii. 34	5	2.28	24.3	3.5	20.4	5. xii. 34
	7. i. 35	40	—	91.6	2.7	22.0	—	—	8. i. 35	28	—	31.2	4.7	21.0	—
	6. ii. 35	59	—	33.4	3.3	27.6	—	—	8. ii. 35	49	—	25.2	4.4	21.0	—
	12. iii. 35	82	3.05	33.9	2.5	23.6	—	—	12. iii. 35	75	2.40	23.7	2.7	19.6	—
	8. iv. 35	101	2.78	165.6	3.3	18.2	—	—	9. iv. 35	94	2.53	21.8	3.1	21.0	—
4707	10. xii. 34	7	2.63	15.0	4.0	28.2	1. xii. 34	4703	3. xii. 34	0	2.50	12.0	4.2	24.0	5. xii. 34
	7. i. 35	27	—	14.5	2.9	18.6	—	—	8. i. 35	26	—	17.4	4.2	25.4	—
	8. ii. 35	45	—	16.4	3.6	14.6	—	—	6. ii. 35	47	—	12.4	4.2	19.8	—
	13. iii. 35	57	2.65	11.4	5.1	28.0	—	—	13. iii. 35	71	2.18	36.6	3.6	21.0	—
	8. iv. 35	68	2.55	21.0	4.6	29.0	—	—	9. iv. 35	87	2.33	10.4	2.5	21.0	—
4702	3. xii. 34	0	2.65	14.3	3.9	21.6	11. xii. 34	4710	3. xii. 34	0	2.10	19.4	3.2	10.6	27. xii. 34
	7. i. 35	20	—	21.8	2.3	—	—	—	8. i. 35	10	—	44.1	1.9	17.0	—
	8. ii. 35	44	—	15.4	2.2	22.6	—	—	8. ii. 35	29	—	21.0*	—	18.4	—
	13. iii. 35	69	2.55	29.2	3.4	20.8	—	—	12. iii. 35	56	2.50	44.2	4.6	18.6	—
	8. iv. 35	87	2.48	37.0	3.2	24.8	—	—	9. iv. 35	77	2.55	36.1	3.5	14.2	—
4708	10. xii. 34	6	2.93	13.3	4.4	—	1. xii. 34	4713	10. xii. 34	22	2.43	101.1	3.4	23.6	8. xi. 34
	7. i. 35	27	—	19.1	5.0	23.0	—	—	8. i. 35	43	—	119.0*	—	12.0	—
	8. ii. 35	47	—	18.2	4.8	23.0	—	—	6. ii. 35	61	—	133.4	3.0	23.6	—
	12. iii. 35	68	3.05	34.5	3.5	23.2	—	—	12. iii. 35	85	2.55	156.4	2.3	21.2	—
	8. iv. 35	88	2.98	44.6	4.7	21.8	—	—	9. iv. 35	105	2.58	159.1	4.0	20.8	—
4705	10. xii. 34	15	2.55	23.5	3.6	22.0	21. xi. 34	4712	10. xii. 34	5	2.68	10.7	3.7	—	4. xii. 34
	7. i. 35	37	—	55.8	1.7	14.4	—	—	8. i. 35	26	—	10.5	4.0	20.6	—
	6. ii. 35	58	—	26.4	1.9	19.0	—	—	8. ii. 35	46	—	8.6	4.2	22.2	—
	13. iii. 35	81	2.48	31.2	2.3	22.0	—	—	13. iii. 35	68	2.68	13.6	3.2	23.8	—
	8. iv. 35	99	2.53	17.2	2.3	—	—	—	9. iv. 35	87	2.90	9.0	5.8	—	—
646	3. xii. 34	Male	3.18	17.2	5.0	10.0	3. xii. 34	581	3. xii. 34	Male	3.55	12.3	3.6	9.5	—
	6. ii. 35	"	—	10.0	2.2	12.2	—	—	8. i. 35	"	—	12.1	4.3	11.3	—
	15. iii. 35	"	—	3.9	3.8	10.2	—	—	6. ii. 35	"	—	8.4	2.8	12.5	—
	11. iv. 35	"	—	5.6	1.9	11.6	—	—	15. iii. 35	"	—	4.6	3.7	10.9	—
	—	"	—	—	—	—	—	—	11. iv. 35	"	—	5.3	2.6	10.8	—
566	10. xii. 34	"	2.85	—	—	—	10. xii. 34	518	10. xii. 34	"	3.23	—	—	—	—
	8. i. 35	"	—	12.1	4.3	10.0	—	—	8. i. 35	"	—	11.5	4.1	9.0	—
	8. ii. 35	"	—	—	2.3	12.0	—	—	15. iii. 35	"	—	7.4	3.2	11.8	—
	16. iii. 35	"	—	5.3	3.6	11.6	—	—	11. iv. 35	"	—	7.7	3.4	11.2	—
	11. iv. 35	"	—	9.8	2.2	12.2	—	—	—	"	—	—	—	—	—

## EXPERIMENT III

The experiments described suggested that egg-laying under conditions believed to be normal is associated in general with increases in serum phosphatase activity. Auchinachie and Emslie, however, did not consider that their results could be put forward in support of this view, and the nature of the variations encountered among laying birds in the present experiment demonstrates the difficulty of interpreting serum phosphatase values in the fowl. Auchinachie and Emslie did suggest, however, that high phosphatase values might have been found in laying birds kept on a low calcium ration in certain mineral metabolism experiments carried out in this laboratory (9, 10). At the time of these experiments the necessary blood analyses could not conveniently be made, but at the conclusion of a somewhat similar mineral metabolism experiment carried out in 1934 this point was open to investigation. A very high value (184 units) was secured from a pullet which had been laying on a low calcium ration, whereas the values for two birds (6.4 and 17.4 units) which had been laying on the same ration supplemented with 5 per cent.  $\text{CaCO}_3$  fell within the range for "normal" laying birds which has been found in this laboratory.

In order to secure a clearer picture, four fresh White Wyandotte pullets, comparable in all respects with those used in this particular experiment, were fed with the same rations and serum phosphatase determinations were made at suitable intervals.

The rations used had the following composition:

	High CaO content	Low CaO content
Bran	20 parts	20 parts
Pollards	40 "	40 "
Sussex ground oats	10 "	10 "
Fish meal	5 "	5 "
Extracted soya-bean meal	10 "	10 "
Maize meal	51 "	56 "
Ground whole wheat	51 "	56 "
Sodium chloride	1 "	1 "
Fortified cod-liver oil	1 "	1 "
Calcium carbonate	10 "	—
CaO	3.100 %	0.365 %
$\text{P}_2\text{O}_5$	0.930 %	0.979 %

The low calcium ration was fed to pullets 1 and 2, and the high calcium ration to pullets 3 and 4.

The experimental results are set out in Fig. 1 and demonstrate a large increase in serum phosphatase in the case of pullets 1 and 2 on the low calcium ration as compared with pullets 3 and 4 on the high calcium ration. The increase was associated with laying, and confirms the cor-

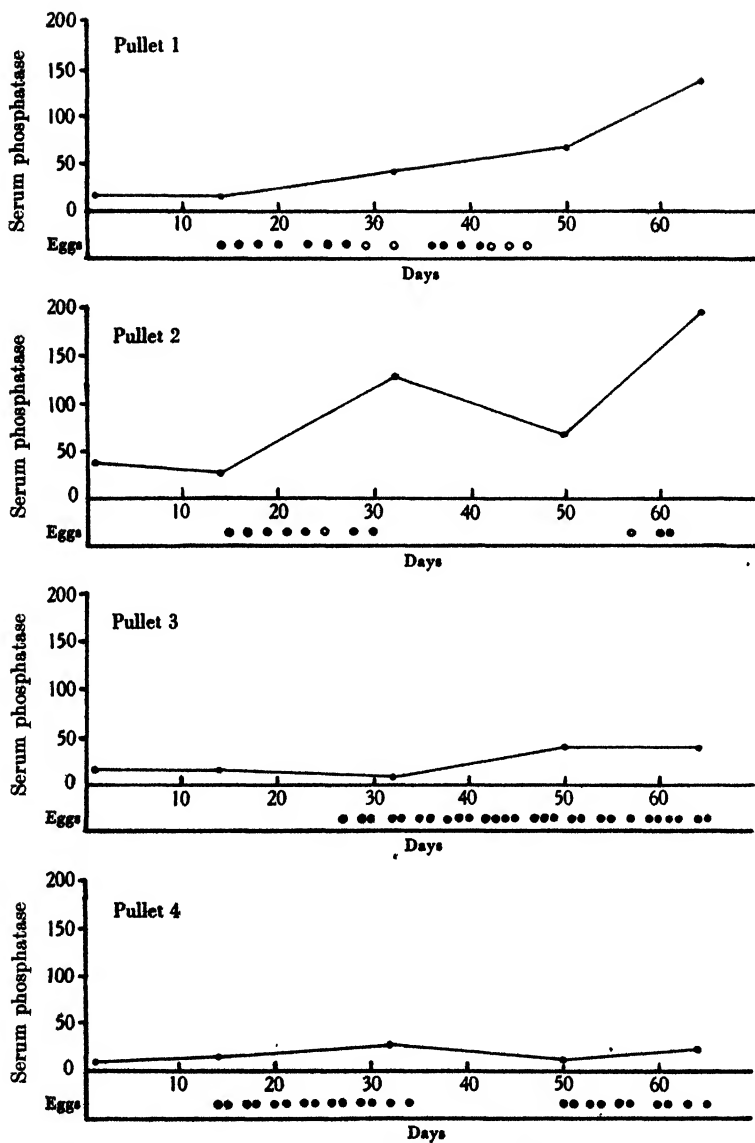


Fig. 1. Effect of calcium deficiency on serum phosphatase of laying pullets.  
(○ denotes soft-shelled egg.)

rectness of the suggestion made by Auchinachie and Emslie. It will be noted that the values for the birds receiving the high calcium ration are of the same order during laying as those found in the laying flock examined in the course of the preliminary survey.

It is probable that body reserves of calcium were being drawn upon for shell formation in the case of pullets 1 and 2. Their tibia-ash values at the end of the experiment were as follows:

Pullet No.	Ration	Tibia ash %
1	Low calcium	51.4
2	"	51.1
3	High calcium	60.5
4	"	60.3

These figures could also be accounted for by a greater storage of calcium and phosphorus in the skeletons of pullets 3 and 4.

The following tentative interpretation of the data submitted in Fig. 1 may now be made. The serum phosphatase values of the pullets receiving the high calcium ration were high, but nevertheless of the same order as those recorded for "normal" laying birds in the other experiments. (For the present the meaning of these increases over the values for non-laying pullets and cocks may be left aside.) The values for the birds on the low calcium ration displayed an increase of a different order, and very high phosphatase values were recorded. This very large increase, it is suggested, is an indication of attack on skeletal reserves of calcium for shell formation. It is conceivable that other sources may have contributed towards bringing about these great increases. Birds laying on such low calcium rations display an increased phosphorus excretion at the time of shell formation, and this is probably excreted via the kidneys. If phosphatase is concerned in phosphate excretion by the kidney<sup>(5)</sup>, then increased kidney phosphatase activity during laying might also contribute towards the increase in serum phosphatase.

#### EXPERIMENT IV

This experiment was carried out in order to secure figures for the serum phosphatase of rachitic chickens.

Two groups of day-old chicks were placed in brooder houses from which all direct sunlight was excluded. One group (Group B) was reared on the rachitogenic diet suggested by McGowan and Emslie<sup>(16)</sup>:

Yellow maize ...	...	...	75 parts
Dried skim milk ...	...	...	10 parts
Dried yeast ...	...	...	10 parts
NaCl ...	...	...	0.5 part
CaCO <sub>3</sub> ...	...	...	6 %



The other group (Group A) received the same diet but with the addition of 2 per cent. cod-liver oil; the cod-liver oil was increased to 4 per cent. at 3 weeks of age. Representative birds were removed for blood analysis and histological examination of the bones at 3, 6 and 9 weeks of age. Group A comprised thirty-six birds at the outset; twenty were removed for analysis, and there were four deaths. Group B

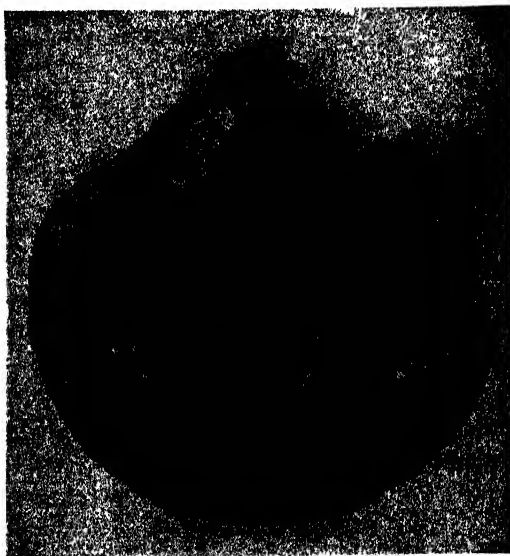


Fig. 2. No. E 4101; 6 weeks old; control group (Group A). Well-defined zone of hypertrophied cartilage cells, with fully calcified spongiosa. Normal bone formation. Tibia ash: 43.5 per cent. Serum phosphatase: 44.7 units. (N.B. The bones from the control group A were not usually so perfectly ossified as those of No. E 4101, and some tended to approach the condition seen in Fig. 3. These facts demonstrate the correctness of the view that Group A as a whole was slightly rachitic, some of the birds being protected and some not.)

The tibio-tarsus was fixed in formaldehyde, and sections cut longitudinally through the distal end by means of a freezing microtome. The sections were stained with silver nitrate; the staining of similar sections by haematoxylin-eosin often helps in the interpretation of the sections stained by silver nitrate.

comprised thirty-seven birds at the outset; twenty-one were removed, and there were sixteen deaths. The results obtained by McGowan and Emslie(16) would lead one to expect normal bone development in the case of Group A and severe rickets in Group B. In actual fact slight clinical signs of rickets were observed in Group A at 3 weeks, and the cod-liver oil was increased from 2 to 4 per cent. of the ration. Group A subsequently appeared normal except that one bird went off its legs at 8 weeks. Histological examination of the bones, however, combined with

tibia-ash determinations, revealed that even at 9 weeks many of the birds in Group A were more or less rachitic, while others were to all intents normal.

The birds in Group B all developed very severe rickets, and at 8 weeks all the surviving birds had gone off their legs.

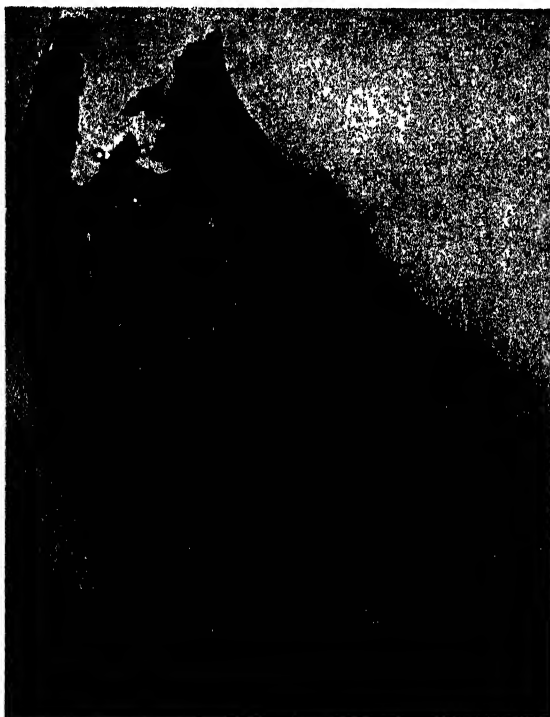


Fig. 3. No. C 4104, 9 weeks old; rachitic group (Group B). There is a wide zone of hypertrophied cartilage cells in which traces of ossification are present. The bone is slightly deformed and presents a picture of severe rickets. Tibia ash: 25.9 per cent. Serum phosphatase: 134.5 units.

The relevant data for this experiment are set out in Table IX. In view of what has been said it is not surprising to find that serum phosphatase was somewhat higher and serum inorganic phosphorus considerably lower than "normal" at 3 weeks in Group A. Serum phosphatase had decreased by 6 weeks, and had decreased still further by 9 weeks; the serum phosphatase of Group A thus followed the "normal" trend, but at a higher level.

These facts are probably related to the presence of a certain degree

Table IX. *Serum phosphatase of rachitic birds*

Group	Normal*			Group A (control)			Group B (rachitic)		
	21	42	63	21	42	63	21	42	63
Age (days) ...	21	42	63	—	363	457	—	301	343
Live weight (gm.)	130	350	670	—	(4)	(5)	—	(3)	(6)
Serum inorg. P (mg./100 ml.)	7-8	8-9	7-8	3.3 (5)	3.8 (4)	3.2 (5)	3.0 (5)	2.4 (3)	3.4 (6)
Serum Ca (mg./100 ml.)	12-13	12-13	10-11	15.3 (5)	15.5 (4)	14.3 (5)	11.4 (5)	12.8 (3)	12.9 (6)
Serum phosphatase (units)	60	40	35	109.0 (5)	66.5 (4)	57.9 (5)	137.1 (5)	199.9 (3)	185.4 (6)
Tibia ash (%)	42	44	44	36.1 (7)	42.0 (3)	37.3 (5)	31.5 (9)	25.9 (3)	28.4 (5)

\* Interpolated from Tables VI and VII.

*Note.* The figures in brackets indicate the number of observations. There was considerable variation in the serum phosphatase values for Group B; in general the degree of rickets appeared to be greater the greater the serum phosphatase.

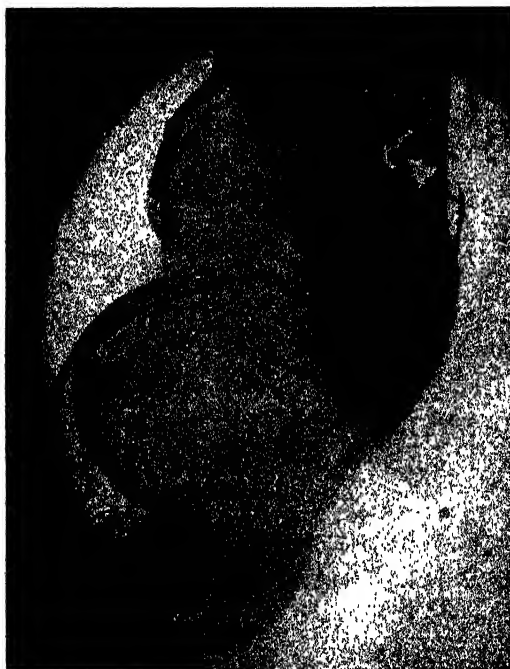


Fig. 4. No. C 4092; 9 weeks old; rachitic group (Group B). There is very extensive proliferation of completely uncalcified cartilage, and the bone is badly deformed. The bird had been "off its legs" for some time. Very severe rickets. Tibia ash: 24.6 per cent. Serum phosphatase: 256.4 units.

of rickets in many cases in Group A even at 6 and 9 weeks, although clinical signs were detected in only one instance after 3 weeks. It will be noted that serum inorganic phosphorus in Group A was lower than normal throughout, and serum calcium was extraordinarily high.

The serum phosphatase values in Group B were very high at 3 weeks, had risen still higher at 6 weeks, and displayed very little tendency to decrease even at 9 weeks. Severe rickets was present in all these birds after 4-5 weeks.

### SUMMARY

1. Some observations on the serum phosphatase of the domestic fowl are described. Laying hens, held to be normal birds, had higher and more variable serum phosphatase than cocks. No great difference in serum phosphatase could be found between laying hens and hens in a period of suspended egg production. Pullets from another source and which had never laid gave values comparable with the value for cocks.

2. The serum phosphatase of chicks shows a rapid increase to a maximum at about 10-12 days after hatching, followed by an equally sharp fall to a lower level at 3 weeks. Thereafter, the values for male birds fall regularly until maturity. The values for female birds correspond closely with the values for male birds until the onset of laying, when they increase again, but to a varying degree in different individuals.

3. An attempt to reduce the range of variation of serum phosphatase in laying birds by administration of 1 ml. per bird per diem radiostol B.D.H. (3000 international units vitamin D per ml.) was not successful.

4. Serum phosphatase showed a much greater increase during laying in pullets receiving a low calcium ration (0.37 per cent. CaO) than in pullets receiving the same ration supplemented with calcium carbonate (3.10 per cent. CaO).

5. Serum phosphatase is greatly increased in chicks suffering from rickets.

The author finds it a pleasure to record his indebtedness to the Director and Staff of the Poultry Research Institute, Hillsborough, County Down, for their co-operation, without which this work would have been impossible. He also desires to thank Dr G. P. McCullagh, of the Pathology Department, Queen's University of Belfast, for his help in making the histological examination of the bones.

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# THE FERTILIZER VALUE OF SOME CONCENTRATED MATERIALS, PARTICULARLY UREA AND GUANIDINE AND THEIR NITRATES AND PHOSPHATES<sup>1</sup>

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(With Two Text-figures)

## INTRODUCTION

DURING recent years considerable attention has been focused on the use of high analysis or concentrated fertilizers. The substances most widely employed at present are mono- and di-ammonium phosphates, ammonium nitrate, urea and potassium nitrate. Besides these, there are a number of other substances which could be produced commercially for use as fertilizers, notably compounds of urea and of guanidine. The aim of the experiments described in this paper was to study the fertilizer value of the nitrogen in certain N- and NP-carrying materials. The substances studied and their analyses were as follows:

	% composition			
	Theoretical		Actual in samples used	
	N	P <sub>2</sub> O <sub>5</sub>	N	P <sub>2</sub> O <sub>5</sub>
Urea nitrate	34.15	—	34.15	—
Urea phosphate	17.72	44.94	17.60	44.90
Guanidine carbonate	46.67	—	46.67	—
Guanidine nitrate	45.90	—	45.70	—
Guanidine phosphate	38.89	32.87	36.00	31.60
Phospham	46.67	118.33	39.10	115.20
Phosphorus nitride	42.94	130.68	44.80	126.70

The only substances in the above list which call for comment at the present juncture are phospham and phosphorus nitride. Phospham (PN<sub>2</sub>H) is a white, non-volatile solid, insoluble in water. It is not affected by dilute hydrochloric or sulphuric acids, but when moistened with water

<sup>1</sup> This paper is based on part of a "Thesis approved for the Degree of Doctor of Philosophy in the University of London".

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and heated it decomposes to give ammonia and metaphosphoric acid, i.e. ammonium metaphosphate. It is not decomposed by dry carbon dioxide, but when heated with alkali carbonates it gives off carbon dioxide and ammonia, and alkali orthophosphates are formed. It is important to note that on hydrolysis phospham yields *meta*-phosphoric acid which may not have the same fertilizer value as *ortho*-phosphoric acid<sup>(1)</sup>. Phosphorus nitride ( $P_3N_3$ ) is a powder, the colour of which may vary from white to red, according to the time it has been heated above  $850^{\circ}\text{C}$ . It is said to be chemically inactive at ordinary temperature. Boiling water slowly converts it into ammonia and orthophosphoric acid, i.e. into ammonium orthophosphates.

### LITERATURE ON FERTILIZER VALUE OF UREA AND GUANIDINE SALTS

Urea is hydrolysed in soil into ammonium carbonate, the ammonia of which is either nitrified or may be directly absorbed by certain plants. It is also possible that under certain conditions urea may be directly absorbed by the plants. The literature on the manurial value of urea is reviewed in detail by Sinclair<sup>(2)</sup>, who concludes that the evidence indicates that urea is comparable in effectiveness with ammonium sulphate, and may be especially suitable for certain crops.

The literature on urea nitrate is very meagre, whilst no reference could be found to any fertilizer experiments with urea phosphate. Münter & Schneidewind<sup>(3)</sup> report the results of a number of experiments with urea nitrate on various crops. On an average, the results with urea nitrate were somewhat better than those with urea. The individual results of pot and field experiments by Haselhoff *et al.*<sup>(4)</sup>, in which urea nitrate was compared with other forms of nitrogen, are very variable, but the average results indicate that urea and urea nitrate were comparable in effectiveness.

Guanidine is the imide of carbamide (urea), and on hydrolysis gives urea and ammonia. The literature on the fertilizer value of guanidine salts is scanty, and the evidence contained therein conflicting. Hutchinson & Miller<sup>(5)</sup> concluded from the results of water culture experiments on peas, that guanidine hydrochloride (80 p.p.m. N) was not toxic, but was utilized only slightly as the sole source of nitrogen. Schreiner & Skinner<sup>(6)</sup>, also working with water cultures, and using wheat, maize, cowpeas and potatoes as test plants, found that guanidine carbonate (12 p.p.m. N) was toxic in the presence but not in the absence of nitrate. In pot culture experiments on a sandy soil, Wagner<sup>(7)</sup> found with oats that, although guanidine nitrate produced some increase in yield, the increase

was much less than that obtained with urea nitrate and sodium nitrate. During early growth, injurious effects with guanidine nitrate were noted. The results of pot experiments conducted by Hiltner<sup>(8)</sup> over a number of years are difficult to interpret. In one year guanidine nitrate had an injurious effect upon the growth of oats, but markedly increased the growth of mustard. Hiltner found a large residual effect in the second year after applying guanidine nitrate. Kappen<sup>(9)</sup> found that guanidine nitrate produced an almost negligible effect on mustard in the field in the first year, but that it had a marked after-effect. From the results of five years' field experiments Haselhoff<sup>(10)</sup> concluded that guanidine carbonate and nitrate were inferior to a number of inorganic nitrates and ammonium salts. The only field experiment carried out in Great Britain on guanidine salts was at Rothamsted in 1919, the crop grown being winter oats, and the nitrogen being applied as a top dressing (34.5 lb. N per acre)<sup>(11)</sup>. The average results for grain, expressed as percentages of the average yields obtained with ammonium and nitrate fertilizers, were: guanidine nitrate 103, guanidine sulphate 90.4, guanidine carbonate 90.2, and no nitrogen 81.

#### OUTLINE OF WORK

To obtain further information on the fertilizer value of the substances enumerated on p. 509, pot culture experiments were conducted with barley and with mustard on acid and neutral soils. Laboratory experiments also were conducted to study the relative rates of nitrification of ammonium, urea and guanidine nitrogen. Since the mustard experiment showed larger differences between forms of nitrogen than did the barley experiment, the changes occurring after the addition of ammonium, guanidine and urea nitrogen to the acid and neutral soils used in the mustard experiment were followed in some detail.

#### BARLEY POT CULTURE EXPERIMENTS

##### *Details of experiments*

These experiments were conducted in pots (14½ in. deep × 7 in. diameter) with two medium loam soils from different fields on Jealott's Hill Farm. Each soil was air-dried and passed through a ¼ in. sieve, and was then mixed with one-half of its own weight of sand. The resultant potting media had pH values of 5.7 and 7.5 respectively. The following fertilizer treatments were compared in sixfold replication: (i) no nitrogen, (ii) ammonium sulphate, (iii) urea, (iv) urea nitrate, (v) urea phosphate,



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(vi) guanidine carbonate, (vii) guanidine nitrate, (viii) guanidine phosphate, (ix) phospham and (x) phosphorus nitride, all nitrogenous substances being applied at a rate equivalent to 0.1578 g. N per pot. All treatments except (v), (viii), (ix) and (x) were given 0.4026 g.  $P_2O_5$  per pot as monocalcium phosphate, this amount being based on the phosphate content of the urea phosphate treatment. In this last treatment no further phosphate was added, while in treatment (viii) (guanidine phosphate) 0.2641 g.  $P_2O_5$  was added as monocalcium phosphate to bring the total  $P_2O_5$  content to 0.4026 g. In (ix) and (x) no further phosphate was added; the equivalent total  $P_2O_5$  contents in these two treatments were 0.4649 and 0.4463 g.  $P_2O_5$  per pot respectively. All treatments were given a dressing of potassium sulphate at the rate of 0.3945 g.  $K_2O$  per pot. The fertilizers were weighed out separately for each pot and were incorporated with the whole of the soil/sand mixture (25 lb.) in each pot.

The moisture content of the medium was brought to 15 per cent, and thirty seeds of barley (variety Opal) were sown per pot. The seedlings were thinned to eight per pot about a month after sowing. At harvest, the straw was cut off at pot level.

### *Yields of grain and straw*

From the results given in Table I, it will be seen that:

(i) applying part of nitrogen as nitrate was of no advantage on either soil,

(ii) on the neutral soil the yields of grain and the numbers of ears were significantly greater where the nitrogen and phosphate applied were in chemical combination than where they were applied in separate compounds,

(iii) ammonium sulphate and urea salts gave significantly better results than did guanidine salts on acid soil but not on neutral soil,

(iv) on the acid soil<sup>1</sup> guanidine nitrate and phosphate gave significantly better results than did guanidine carbonate,

(v) the results with ammonium sulphate did not differ significantly from those with urea on either soil,

(vi) on the acid soil, urea and urea phosphate gave significantly better results than urea nitrate, and

(vii) phospham and phosphorus nitride were of little use as fertilizers; that the lack of response to phospham was not due to lack of orthophosphate was shown by the fact that extra pots treated with

<sup>1</sup> The interaction of form of cation  $\times$  form of anion was significant for yields of grain and straw and for numbers of ears on the acid soil but not on the neutral soil.

phospham + monocalcium phosphate gave practically the same results as phospham alone.

Table I. *Barley experiment—average results per pot*

	Acid soil			Neutral soil		
	Grain g.	No. of ears	Straw g.	Grain g.	No. of ears	Straw g.
No nitrogen	8.01	14.7	7.10	8.82	16.2	10.17
Ammonium sulphate	14.10	21.3	14.35	13.33	19.7	15.13
Urea	13.94	20.2	13.87	13.99	20.2	14.33
Urea nitrate	12.98	18.8	12.75	13.43	20.3	14.47
Urea phosphate	14.10	19.7	14.18	15.02	21.5	15.03
Guanidine carbonate	11.43	16.7	11.58	12.86	18.7	13.47
Guanidine nitrate	13.46	19.2	13.30	13.30	20.3	14.27
Guanidine phosphate	12.78	18.8	12.23	14.89	21.5	14.30
Phospham	8.04	14.8	8.98	10.91	17.2	11.90
Phosphorus nitride	8.30	16.2	10.53	11.21	17.7	13.18
Mean	11.71	18.0	11.89	12.77	19.3	13.63
Standard error	0.416	0.586	0.514	0.675	0.814	0.626
<i>Average results</i>						
Cation:						
Urea	13.67	19.6	13.60	14.15	20.67	14.61
Guanidine	12.56	18.2	12.37	13.85	20.17	14.01
Anion:						
Non-nutrient	12.67	18.45	12.73	13.43	19.45	13.90
Nitrate	13.22	19.00	13.03	13.37	20.30	14.37
Phosphate	13.44	19.25	13.21	14.96	21.50	14.67

Weights of grain and straw are oven-dry weights (98° C.).

## MUSTARD POT CULTURE EXPERIMENTS

### *Details of experiments*

The experiments were conducted in acid and neutral media. Instead of obtaining acid and neutral soils directly from suitable fields, as was done for the barley experiments, an acid soil, light in texture, was obtained from Beenham, Berks. The soil was air-dried and passed through a  $\frac{1}{4}$  in. sieve and was mixed with one-half of its own weight of sand. The neutral medium used in the pots was prepared by adding the requisite amount of  $\text{CaCO}_3$  (determined by direct experiment) to the acid soil/sand mixture, and keeping it in a moist condition for some weeks.

The following fertilizer treatments<sup>1</sup> were compared in fourfold replication: (i) no nitrogen, (ii) ammonium sulphate, (iii) ammonium nitrate, (iv) mono-ammonium phosphate, (v) urea, (vi) urea nitrate, (vii) urea phosphate, (viii) guanidine carbonate, (ix) guanidine nitrate, (x) guanidine

<sup>1</sup> Phospham and monocalcium phosphate were not included since they gave such poor results with barley.

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dine phosphate and (xi) calcium nitrate, all nitrogenous substances being given at a rate equivalent to 0.3516 g. N per pot. The same amount of  $P_2O_5$  (0.7032 g.) was applied to all pots. In treatments (i), (ii), (iii), (v), (vi), (viii), (ix) and (xi) all the phosphate was applied as superphosphate (13.73 per cent water soluble  $P_2O_5$ ). In treatment (iv) all the phosphate was applied as mono-ammonium phosphate, and the extra nitrogen required to reduce the N :  $P_2O_5$  ratio to 1 : 2 was applied as ammonium sulphate (0.2130 g. N). Similarly, all the phosphate in (vii) was applied as urea phosphate, extra urea (0.0760 g. N) was added to reduce the N :  $P_2O_5$  ratio to 1 : 2. In (x) all the nitrogen was applied as guanidine phosphate, and superphosphate (0.3946 g.  $P_2O_5$ ) was added to raise the N :  $P_2O_5$  ratio to 1 : 2. All treatments were given a dressing of potassium chloride at the rate of 0.3516 g.  $K_2O$  per pot.

The fertilizers were weighed out separately for each pot and were mixed with the top 6 in. of soil/sand mixture only, and not with all the soil/sand mixture in the pots as in the barley experiment. The moisture content of the pots was maintained at 15 per cent. Sixteen seeds of white mustard were sown per pot. Germination was better on the neutral than on the acid soil, but there were no appreciable differences between treatments. The seedlings were thinned to 8 per pot 1 week after sowing. The first crop was harvested after 1 month. The whole plants were pulled up, the tops cut off, and the roots washed free from soil. The soil was levelled and the pots were resown, thinned, harvested, etc., as for the first crop.

### *Yields of dry matter*

From the results given in Table II it will be seen that:

(i) in contrast with barley, applying part or all of the nitrogen as nitrate markedly increased yields on the acid soil,

(ii) on the neutral soil the second crop yields were significantly greater where nitrogen and phosphorus were applied in chemical combination than where they were applied in separate compounds,

(iii) the first crop yields with urea salts were significantly greater than those with ammonium salts in both soils,

(iv) the first crop yields with calcium nitrate were greater on the acid soil but less on the neutral soil than those with urea salts,

(v) guanidine carbonate and phosphate did not significantly affect the first crop yields on either soil, but they gave increases in the second crops,

(vi) although guanidine nitrate gave a large and significant increase

Table II. *Mustard experiment*

	Acid soil						Neutral soil					
	First crop			Second crop			First crop			Second crop		
	Total yield			Total yield			Total yield			Total yield		
	Yield g.	% N	N uptake g.	Yield g.	% N	N uptake g.	Yield g.	% N	N uptake g.	Yield g.	% N	N uptake g.
No nitrogen	3.18	4.75	0.151	2.68	3.73	0.100	9.08	2.61	0.255	3.60	2.70	0.100
Ammonium sulphate	2.45	5.01	0.123	3.29	4.57	0.150	10.64	3.12	0.364	4.56	3.03	0.138
Ammonium nitrate	6.18	4.80	0.207	3.53	4.15	0.147	10.99	3.67	0.403	4.20	2.91	0.125
Mono-ammonium phosphate	4.80	4.37	0.234	3.04	4.11	0.131	10.74	3.92	0.421	5.20	2.82	0.146
Urea	5.53	4.42	0.245	3.40	4.12	0.140	8.93	3.75	0.425	4.18	2.85	0.119
Urea nitrate	7.10	4.41	0.313	3.31	3.99	0.132	11.74	3.68	0.432	4.35	3.01	0.131
Urea phosphate	3.58	5.17	0.185	3.90	4.30	0.165	11.79	3.72	0.439	4.95	2.73	0.135
Guanidine carbonate	2.86	4.84	0.131	3.45	1.13	0.143	8.88	3.26	0.290	1.93	2.91	0.143
Guanidine nitrate	4.79	5.04	0.241	3.60	4.31	0.164	9.55	3.35	0.320	5.98	2.87	0.172
Guanidine phosphate	2.20	4.70	0.103	3.09	4.21	0.130	8.56	3.70	0.317	6.41	2.82	0.161
Calcium nitrate	6.58	4.35	0.288	4.18	3.88	0.162	10.53	3.98	0.419	5.08	2.85	0.145
Mean	4.48	4.73	—	3.42	4.16	—	10.35	3.57	—	4.87	2.86	—
Standard error	0.5683	—	—	0.2335	—	—	0.3089	—	—	0.3489	—	—
<i>Average results</i>												
Cation:												
Ammonium	4.46	—	—	3.29	—	—	10.79	—	—	4.06	—	—
Urea	5.40	—	—	3.54	—	—	11.62	—	—	4.49	—	—
Guanidine	3.29	—	—	3.45	—	—	9.00	—	—	5.77	—	—
Anion:												
Non-nutrient	3.62	—	—	3.38	—	—	10.28	—	—	4.55	—	—
Nitrate	6.02	—	—	3.55	—	—	10.76	—	—	4.87	—	—
Phosphate	3.53	—	—	3.34	—	—	10.36	—	—	5.52	—	—

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in first crop yield on the acid soil, its effect on the first crop on the neutral soil was small and insignificant, and

(vii) on the acid soil the first crop yield with ammonium phosphate was significantly greater than that with ammonium sulphate (+super-phosphate).

Table III. *Percentage apparent recovery in crop of added nitrogen*

	Acid soil			Neutral soil		
	1st crop	2nd crop	Total	1st crop	2nd crop	Total
Ammonium sulphate	-8.0	14.5	6.5	31.0	11.0	42.0
Ammonium nitrate	41.5	13.5	55.0	42.0	7.5	49.5
Ammonium phosphate	23.5	10.0	33.5	47.5	13.0	60.5
Urea	27.0	11.0	38.0	48.5	5.5	54.0
Urea nitrate	46.0	9.0	55.0	50.0	8.0	58.0
Urea phosphate	9.5	19.5	29.0	52.0	11.0	63.0
Guanidine carbonate	-5.5	12.0	6.5	10.0	12.0	22.0
Guanidine nitrate	26.0	18.0	44.0	18.5	20.5	39.0
Guanidine phosphate	-13.5	5.0	-8.5	18.0	23.0	41.0
Calcium nitrate	39.0	18.0	57.0	47.0	13.0	60.0

### UPTAKE AND APPARENT RECOVERY OF ADDED NITROGEN

From Tables II and III it will be seen that:

(i) the percentage nitrogen content of the dry matter was much higher on the acid than on the neutral soil,

(ii) on the acid soil the percentage nitrogen content of the first crop was inversely related to yield, and

(iii) despite (ii) above, the uptake of nitrogen was closely related to yield, the apparent recovery of added nitrogen thus being about the same on both soils where part or all of the nitrogen was applied as nitrate, but being higher on the neutral soil for forms of nitrogen other than nitrate.

### NITRIFICATION EXPERIMENTS WITH SOILS USED

#### IN MUSTARD EXPERIMENTS

#### *Technique*

20 g. portions of soil/sand mixture to which the material under examination (urea, guanidine carbonate, etc.) had been added at a rate equivalent to 100 parts of N per million parts of soil, were incubated at 20 per cent moisture content and 25°C. in flasks fitted with cotton-wool plugs. The moisture content of the flasks was readjusted to 20 per cent every 2 weeks. This technique is similar to that described by Crowther & Brenchley(12), except that these authors shook the contents of the flasks to break up the soil, whilst in the experiments described in the

present paper the flasks were not shaken. Ammonia and nitrate were determined in extracts of duplicate flasks per treatment by distillation with MgO and with MgO + Devarda's alloy respectively. Olsen's<sup>(13)</sup> extraction medium was used. Since hydrolysis of urea and guanidine carbonate, under the standard conditions used in the distillations, was small (4 and 1 per cent respectively) no allowance was made for it. The total nitrogen content of the extracted soil (later called "residual" nitrogen) was also determined; the figures are probably accurate to about 10 p.p.m.

The ammonia and nitrate results are shown graphically in Fig. 1. The standard errors of means of two flasks were: nitrate 1.42, ammonia 1.28, ammonia + nitrate 0.98 part of nitrogen per million parts of oven-dry soil.

#### *Ammonia and nitrate*

The results may be summarized as follows:

(i) much larger amounts of nitrate were formed in the neutral soil than in the acid soil,

(ii) on the acid soil, whereas ammonium sulphate significantly depressed the nitrate content, urea gave a significant increase in nitrate; this difference was probably largely due to differences in the effects of ammonium sulphate and urea on soil reaction,

(iii) on the neutral soil although ammonium sulphate gave a large increase in nitrate, the increase with urea was significantly greater, and

(iv) guanidine carbonate gave a small but steady and significant increase in nitrate in the acid soil, but in the neutral soil guanidine carbonate reduced the average nitrate content, a large increase in nitrate in the later stages being outbalanced by an even greater decrease in the early stages.

When considered in the light of these results, the conclusion of Funchess<sup>(14)</sup> that nitrification of guanidine carbonate occurs in acid soils but is inhibited by lime acquires a deeper meaning; it would appear that Funchess may have based his opinion regarding limed soils on results for the critical period between the initial decrease and the final increase in nitrate.

#### *Apparent recovery of added nitrogen*

The average apparent recoveries as ammonia + nitrate of the added nitrogen were:

	Acid soil	Neutral soil
Ammonium sulphate	90	87
Urea	90	98
Guanidine carbonate	7	21

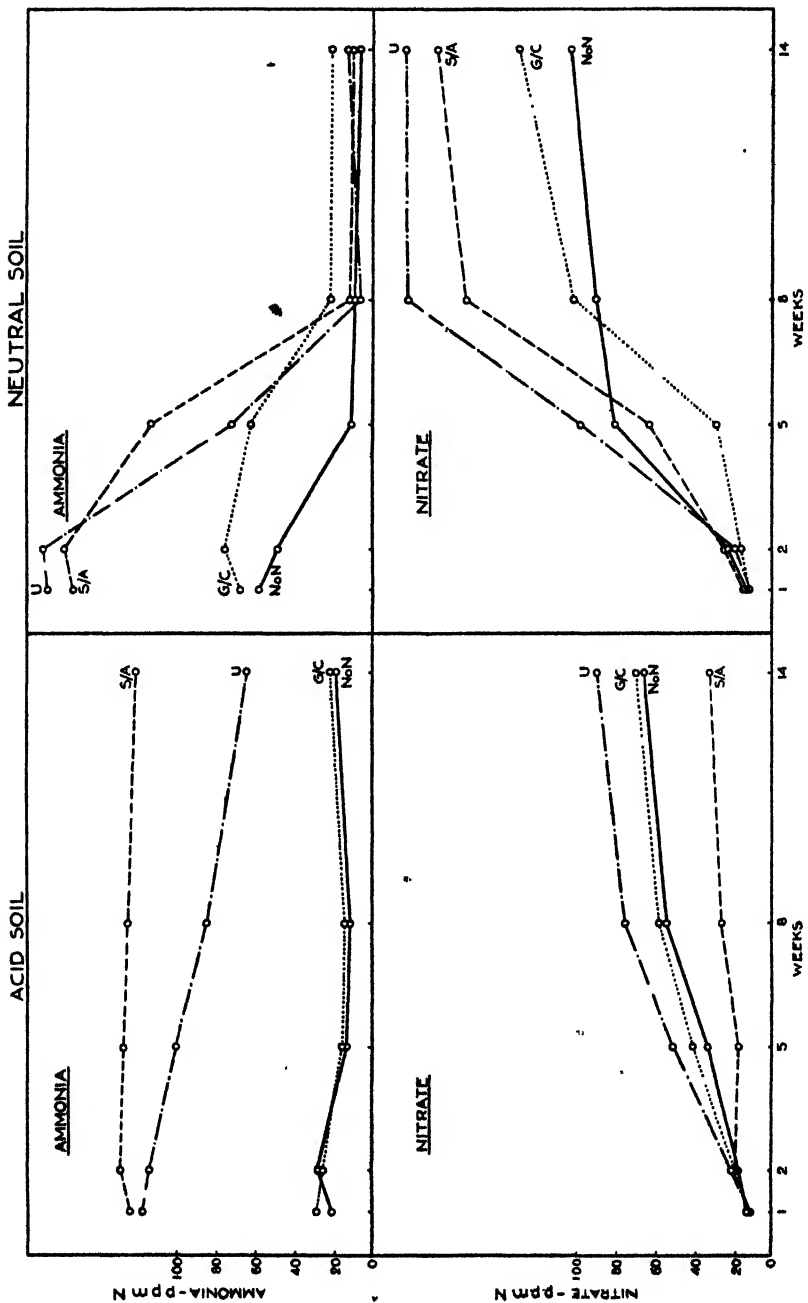


Fig. 1.

The incomplete recoveries with ammonium sulphate on both soils and with urea on the acid soil may have been due to losses of nitrogen or to the fertilizers depressing the rate of ammonification of the soil organic matter; no such effect occurred with urea on the neutral soil. The recovery with guanidine carbonate was low on both soils, but was much greater on the neutral soil than on the acid soil.

*Total soluble nitrogen*

If it is assumed that no loss or gain in total nitrogen content occurred, then the differences between the original nitrogen (1630 p.p.m.) and the "residual" nitrogen contents represent the total soluble nitrogen (inorganic + organic). The values thus obtained, which are given in Table IV, are probably accurate to about 10 or 20 p.p.m.

Table IV. *Total soluble nitrogen—p.p.m. 100 p.p.m. N applied*

	Weeks					Average	Increase due to added N
	1	2	5	8	14		
Acid soil:							
No nitrogen	40	60	70	40	80	60	—
Ammonium sulphate	150	130	210	170	160	160	100
Urea	140	150	190	140	140	150	90
Guanidine carbonate	140	150	160	140	180	150	90
Neutral soil:							
No nitrogen	90	120	180	110	150	130	—
Ammonium sulphate	190	210	240	220	230	220	90
Urea	200	210	240	210	270	230	100
Guanidine carbonate	200	200	220	200	180	200	70

The results show that:

(i) the total soluble nitrogen was much higher on the neutral than on the acid soil, and

(ii) the apparent recoveries, which are given in the last column of the table, are 90–100 per cent except for guanidine carbonate on the neutral soil where the figure is only 70 per cent. This low value, which is due to high values for "residual" nitrogen, indicates that guanidine carbonate had depressed the solubility and ammonification of the organic matter on the neutral soil.

NITRIFICATION EXPERIMENTS WITH ACID SOIL USED  
IN BARLEY EXPERIMENT

Since the only very marked difference between the results of the mustard and barley experiments was in the poorer results with ammonium salts on acid soil with mustard than with barley, the nitrification



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experiment corresponding to the barley experiment was restricted to a study of the behaviour of ammonium sulphate in the acid soil/sand medium used in the barley experiment. The technique adopted was identical with that used in the nitrification experiments with the mustard soils, the ammonium sulphate being added at a rate corresponding to 100 parts N per million parts of soil. The results obtained are given in Table V.

Table V. *P.p.m. N*

	1 week			3 weeks		
	NH <sub>3</sub>	NO <sub>3</sub>	NH <sub>3</sub> + NO <sub>3</sub>	NH <sub>3</sub>	NO <sub>3</sub>	NH <sub>3</sub> + NO <sub>3</sub>
No nitrogen	117	15	132	139	11	150
Ammonium sulphate	201	13	214	225	11	236
Increase due to ammonium sulphate	—	—	82	—	—	86

	6 weeks			8 weeks not inoculated		
	NH <sub>3</sub>	NO <sub>3</sub>	NH <sub>3</sub> + NO <sub>3</sub>	NH <sub>3</sub>	NO <sub>3</sub>	NH <sub>3</sub> + NO <sub>3</sub>
No nitrogen	193	11	204	199	13	212
Ammonium sulphate	263	10	273	278	14	292
Increase due to ammonium sulphate	—	—	69	—	—	80

The fact that no increase in nitrate occurred is surprising since the soil was considerably less acid than the acid mustard soil in which some nitrification occurred.

### DISCUSSION

Certain general aspects of the relationships between the results of the pot experiments and those of the laboratory nitrification experiments need consideration before the results are discussed in detail. One of the most important differences between the results of the barley and mustard pot experiments was that on the acid soils applying part of the nitrogen as nitrate markedly increased the yields of mustard, but not those of barley. The results of the pot experiments with mustard on the acid soil, considered in the light of the laboratory nitrification experiments, can be quite simply explained on the assumption that any nitrate nitrogen absorbed by mustard was very effective and any ammonium nitrogen absorbed was ineffective in increasing yields, and that the uptake of nitrate was very much greater than that of ammonia.

It might be assumed that, because no nitrification occurred in the laboratory in the acid barley soil, ammonium nitrogen was absorbed and effectively utilized in large amounts by barley on the acid soil.

However, nitrification occurs fairly rapidly in the same soil not only in the field but also in samples taken from the field and incubated in the laboratory in a moist condition without being first dried at air temperature<sup>(15)</sup>. The laboratory nitrification experiment described on p. 520 was conducted with a soil which had been stored in an air-dry condition for several months,<sup>1</sup> and this treatment may have had a harmful effect on the nitrifying organisms. The soil used in the pot experiments was also air-dried, but was not stored for more than a few weeks; it is impossible to state whether this resulted in death of most or all of the nitrifying organisms, but, even if it did, some reinfection would probably have occurred during the course of the pot experiments. In a pot experiment conducted earlier on the acid soil used in the mustard experiments (*pH* 5.2 compared with 5.7 for the acid barley soil), calcium nitrate gave much greater increases in yield of barley than did ammonium sulphate. It seems unlikely that barley can use ammonium nitrogen effectively at *pH* 5.7 and ineffectively at *pH* 5.2, and it may, therefore, be argued that some nitrification probably occurred in the acid soil in the barley experiments under consideration.

As far as the neutral soils are concerned, it is possible that both barley and mustard assimilated some ammonium nitrogen, but since nitrification was rapid in both neutral soils, most of the nitrogen may have been absorbed as nitrate. Further, the results of a pot experiment in a medium consisting of calcium bentonite and sand of very poor nitrifying power showed that, even at a *pH* of 7.5, mustard made very little growth with ammonium nitrogen, whereas growth with added nitrate was very good.

The mustard results show certain other interesting features. The percentage nitrogen content of the dry matter was much higher on the acid soil than on the neutral soil; on the acid soil the percentage nitrogen content of the first crop was inversely related to yield which, in turn, was proportional to nitrate supply (Fig. 2). This may have been due to the fact that some ammonia was absorbed by the mustard on the acid soil, but was not balanced by an increase in yield of dry matter such as occurred with nitrate. Further, on the neutral soil the yields and uptake of nitrogen were proportional to the inorganic nitrogen content ( $\text{NH}_3 + \text{NO}_3$ ) in the soil (Fig. 2).

The yields of barley and mustard on neutral soils were significantly greater where nitrogen and phosphorus were applied in chemical com-

<sup>1</sup> The soil used in the mustard pot experiments and in the corresponding nitrification experiments was also air-dried.

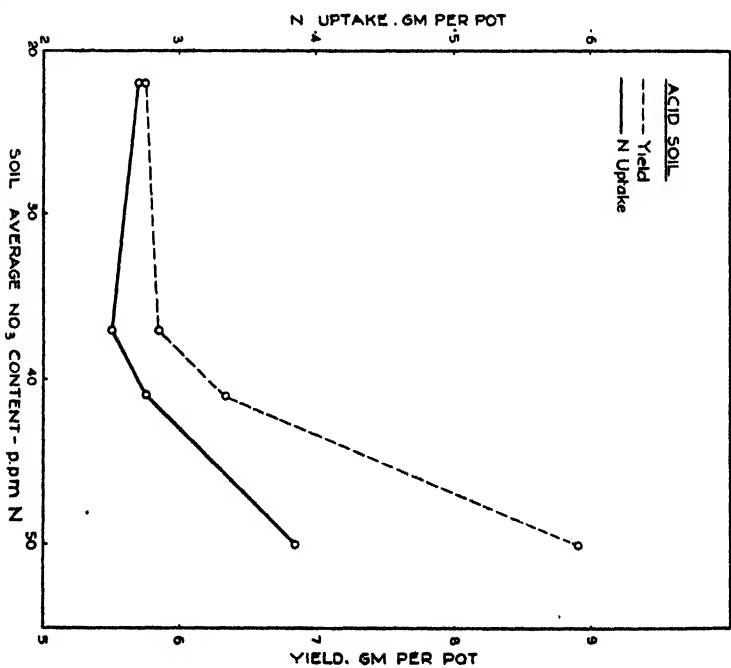
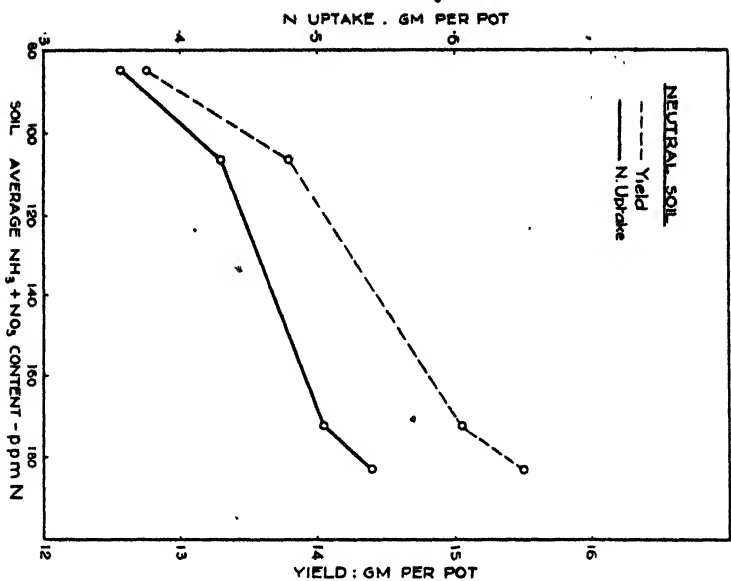


Fig. 2.

bination than where they were applied in separate compounds; with mustard the advantage with NP compounds occurred in the second crop. It may be that the phosphorus of the NP compounds was more available than that of superphosphate. Any differences in availability of phosphorus may not be so much due to differences in amounts fixed in an insoluble form by chemical reaction as to differences in factors, such as ionization constants of the phosphates, which directly or indirectly affect the energy relationships of phosphorus assimilation<sup>(16)</sup>.

The barley and mustard results show that nitrogen applied in the form of guanidine was slow acting, but was more rapid in action on neutral than on acid soils. This agrees with the results of the nitrification experiments in which the hydrolysis of guanidine was quicker and less incomplete on neutral soil. In contrast with much of the published literature, the results afford no definite evidence of any toxic effects of guanidine on plant growth. The failure of guanidine nitrate to increase the first crop yield of mustard on neutral soil is nevertheless surprising since one-quarter of its nitrogen was in nitrate form. Whilst this lack of response to guanidine nitrate may have been partly due to a direct injurious effect of guanidine, it could be explained, at least in part, by a depressing effect of guanidine on nitrification in the early stages such as that which occurred in the laboratory experiment on neutral soil, so that the nitrate part of the guanidine nitrate could do no more than make up the reduction in production of nitrate from the soil organic matter. It would appear that guanidine salts might be useful where a slow acting nitrogenous fertilizer is required. Being more basic than urea, guanidine may enter the absorption complex of soil and be protected against loss by leaching.

Whilst on both acid and neutral soils urea salts gave significantly greater first crop yields of mustard than did ammonium salts, and on the neutral soil urea also gave a significantly greater first crop yield than did calcium nitrate, no such differences occurred with barley. The better results with urea than with ammonium salts on mustard may have been due to a greater rate of nitrification of urea (see Fig. 1), but the reason for the better results with urea than with calcium nitrate is obscure; it may be that urea itself was absorbed by the plants and used more efficiently than nitrate, or urea may have "mobilized" a fraction of the soil organic matter.<sup>1</sup> In this regard some results obtained by Marmoy and the present author are of interest. In experiments with Italian

<sup>1</sup> It is extremely improbable that differences in amounts of soluble salts applied could have affected the results under the conditions obtaining.

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rye-grass in sand culture, urea appeared to have an effect on early growth over and above that which would have been expected from its nitrogen content.

The absence of any significant difference between the effects of urea and ammonium salts on the yields of barley may have been due to any increased nitrification or any "mobilization" of soil organic matter brought about by urea being only temporary and thus having little or no effect on the more slowly growing barley crop; it is also possible that even if urea did result in more nitrogen being available, a smaller amount of available nitrogen may have been sufficient to supply the needs of the crop, at the existing levels of other growth factors.

Two other results remain to be considered. The first is that on acid soil, ammonium phosphate gave a significantly greater first crop yield of mustard than did ammonium sulphate (+ superphosphate); the higher yield with ammonium phosphate was probably due to it having a smaller effect in increasing soil acidity by cation exchange. (The results of a laboratory incubation experiment, which are not quoted here owing to exigencies of space, showed that the rate of nitrification in the acid mustard soil was considerably greater with added ammonium phosphate than with ammonium sulphate and superphosphate.) The second is that on the acid soil urea nitrate gave significantly lower yields of barley than did urea and urea phosphate; one possible explanation of this is that urea nitrate was reduced to urea nitrite, either in the soil or the plant, which decomposed to give gaseous nitrogen.

### SUMMARY AND CONCLUSIONS

A survey of the fertilizer value of certain highly concentrated N and NP materials, particularly salts of urea and of guanidine, which might be used as fertilizers, has been made. The evidence in the literature indicates that whilst guanidine carbonate and nitrate, especially the latter, may under certain conditions be toxic to the growth of certain plants, under other conditions these guanidine salts have considerable fertilizer value which is, however, less than that of ammonium sulphate. Urea nitrate appears to have about the same value as guanidine nitrate. No reference to experiments with guanidine or urea phosphates could be found.

Pot culture experiments were conducted with barley and mustard in acid and neutral soils to study the effects of ammonium, urea and guanidine salts, including their nitrates and phosphates, and of two extremely concentrated materials, phospham ( $\text{PN}_2\text{H}$ ) and phosphorus

nitride ( $P_3N_5$ ). The rate of nitrification of ammonium, urea and guanidine nitrogen were also studied in the laboratory.

There was no significant difference between the effects of ammonium and urea salts on the yields of barley. The yields of mustard with urea salts were, however, significantly greater than those with ammonium salts on both soils and greater than those with calcium nitrate on the neutral soil. The better results on both soils with urea than with ammonium salts may have been due to a greater rate of nitrification of urea. The reason for the greater first crop yield on neutral soil with urea than with calcium nitrate is obscure; it may be that urea itself was absorbed and used more efficiently than nitrate, or urea may have "mobilized" a fraction of the soil organic matter. Urea salts may thus be particularly suitable for use on neutral soils where quick action is required.

Guanidine salts were slower in their action than were ammonium and urea salts. Guanidine salts gave greater increases in yields of barley and mustard on neutral than on acid soils. In contrast with much of the published literature, no direct evidence was obtained of any toxic effects of guanidine salts, but the fact that guanidine nitrate, one-quarter of the nitrogen of which is in nitrate form, did not increase the first crop yield of mustard on neutral soil is suggestive. This lack of response to guanidine nitrate may have been due to a depressing effect of guanidine on nitrification in the early stages. Guanidine salts may prove useful where a slow-acting form of nitrogen is required.

The results show that phospham and phosphorus nitride have very little manurial value. Both were a little more effective on neutral soil than on acid soil, and phosphorus nitride appeared to be somewhat more effective than phospham.

On the neutral soils the yields of barley and mustard were significantly higher where part or all of the phosphate was applied in chemical combination with the nitrogen than where it was applied separately in the form of superphosphate; this difference may have been due to a greater availability of the phosphate of nitrogen-phosphorus compounds.

The results of laboratory nitrification experiments afford a simple explanation of the results of the mustard pot experiments, yields and uptake of nitrogen being related to nitrate content on the acid soil and to inorganic nitrogen (ammonia + nitrate) on the neutral soil. The failure of the acid barley soil to nitrify when incubated in the laboratory, although it was known to nitrify in the field, shows, however, that considerable caution must be exercised in interpreting the results of laboratory nitrification experiments.

## ACKNOWLEDGEMENTS

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# THE TRANSPORT OF WATER THROUGH HEAVY CLAY SOILS. III

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(With Eight Text-figures)

## INTRODUCTION

SOME explanation of the objects of this paper is, perhaps, advisable, since it deals largely, not with heavy clay soils, but with soils of lighter texture. Since, however, it is concerned with the principles of diffusion introduced in papers I and II<sup>(1)</sup>, it should properly be included in the same series.

For some years it has been customary to hold the view that water, when applied to a dry soil either directly or by means of placing wetter soil in contact with the drier, moistens the upper dry soil uniformly to some well-defined depth and then remains stationary, in a state of equilibrium, in spite of the moisture gradient at the lower surface of the moistened layer. In other words, if moisture content be plotted against depth at various times subsequent to a surface application of water, the water front will be observed to advance as a "step", as illustrated by curves I-IV in Fig. 1, reaching a final position of equilibrium, curve IV. Schofield<sup>(2)</sup> has recently sought to account for this behaviour on a basis of the hysteresis loop in the soil-moisture *v.* pressure-deficiency curve first demonstrated by Haines<sup>(3)</sup>. He quotes, from the literature on the subject, four experimental contributions from American authors<sup>(4, 5, 6, 7)</sup>. In discussion it was pointed out that it is quite unnecessary to introduce hysteresis to account for an apparent equilibrium corresponding to non-uniform moisture distribution at the end of some arbitrary and ill-defined interval of time after the application of water. The diffusion theory<sup>(1)</sup> shows that this "equilibrium" is only a stage in an aperiodic phenomenon. It is a well-known feature of aperiodic phenomena in nature, that the nearer they approach the final state the slower the approach becomes, so that a hasty observer might confuse any of the stages of slow progress with the true final state.

In a subsequent reply it was stated<sup>(8)</sup> that the diffusion theory was



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inadequate to account, even approximately, for the moisture distributions observed. It is therefore the purpose of this paper to show three things:

(a) That the diffusion theory is consistent with the observed facts quoted.

(b) That the diffusion theory yields moisture distribution curves which are in very fair agreement with the most reliable of the experimental results, since a satisfactory theory must agree, not only in general features, but in numerical detail.

(c) That the alternative hysteresis theory is untenable, being in conflict with the experiments quoted.

### SURVEY OF THE EXPERIMENTAL EVIDENCE

Before attempting any of the three points (a), (b), (c), it is well to give a summary of the results to be explained. It is convenient to begin with the field observations of Israelsen<sup>(5)</sup> on the results of irrigation. Working with a variety of soil types ranging from a silt loam on fine sandy loam subsoil to clay, he obtained no evidence of a "step" of the type of Fig. 1 (he determined the moisture profiles after only one unspecified interval of time following irrigation), with the exception of the lightest soil. Even here, the "step" was of the nature of a taper as long as the depth of uniform moistening. For the rest of the soils, the gain of moisture was found to decrease with depth from the surface, the effect becoming increasingly marked as the soils studied became heavier. This type of profile is in qualitative agreement with case 2 of paper I<sup>(1)</sup>, in which is worked out the subsequent moisture profiles when the surface is maintained at constant moisture content; this is the simple condition most nearly approximating to the actual treatment, which consisted of a number of light irrigations. The lack of essential data renders numerical calculation impossible. There is no suggestion that such profiles constitute final states of equilibrium; in fact, some years later Israelsen and West<sup>(9)</sup> express their opinion that the downward movement of the moisture must continue until equilibrium is established with the water table.

Table I

Soil fraction	Alway & Clark	Shaw
Gravel	2.84	—
Coarse sand	2.00	—
Medium sand	1.33	—
Fine sand	4.16	70.0
Very fine sand	67.11	
Silt	14.16	11.5
Clay	8.40	8.5

The experiments of Shaw(6) and of Alway & Clark(4) may be considered together, since the mechanical analyses of the soils used show a marked similarity, as will be seen in Table I. Shaw actually carried out experiments with heavier soils, but owing to the slow movement of moisture he considered his results unreliable and did not publish them. This conclusion was a natural one in the circumstances, as the diffusion nature of the movement was not recognized. The experiments of Alway

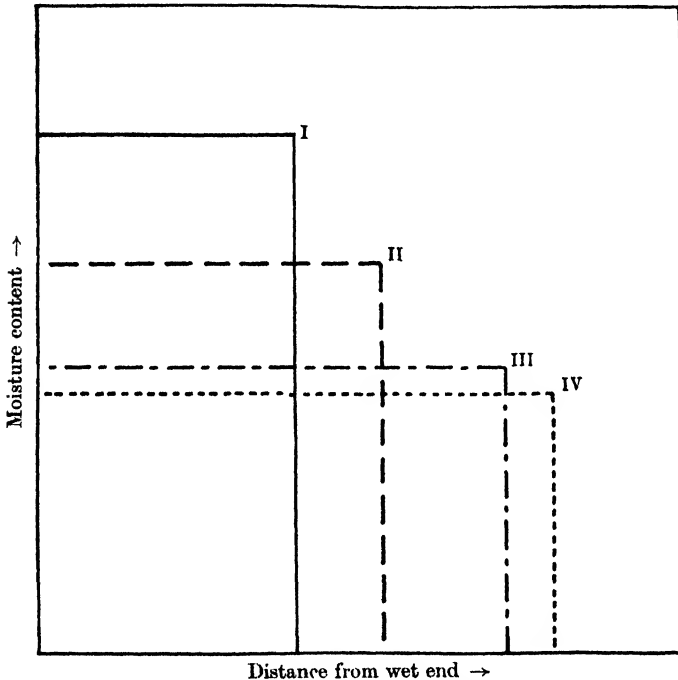


Fig. 1. Progress of water front. Curve I, initial moisture distribution; Curve II, second moisture distribution; Curve III, third moisture distribution; Curve IV, final moisture distribution.

& McDole(4) do not affect the issue, since they obtained no detailed moisture profiles but contented themselves mainly with following the progress of the water front as indicated by the change of soil coloration. In view of Veihmeyer's(7) experience that the line of demarcation between wet and dry soils disagrees seriously with the measured moisture profiles, these results must be considered to be unsatisfactory material for discussion.

We observe at once a discrepancy between the results of Shaw and those of Alway & Clark. These authors apply moisture to a column of

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dry soil (described by the latter as artificial conditions), the former by applying a known mass of water to one end, the latter by placing a known quantity of wet soil in contact with one end. In each case the tubes containing the soil were then sealed to avoid loss by evaporation. Shaw found, by sampling after 25, 54, 89 and 123 days (see his Fig. 4), that

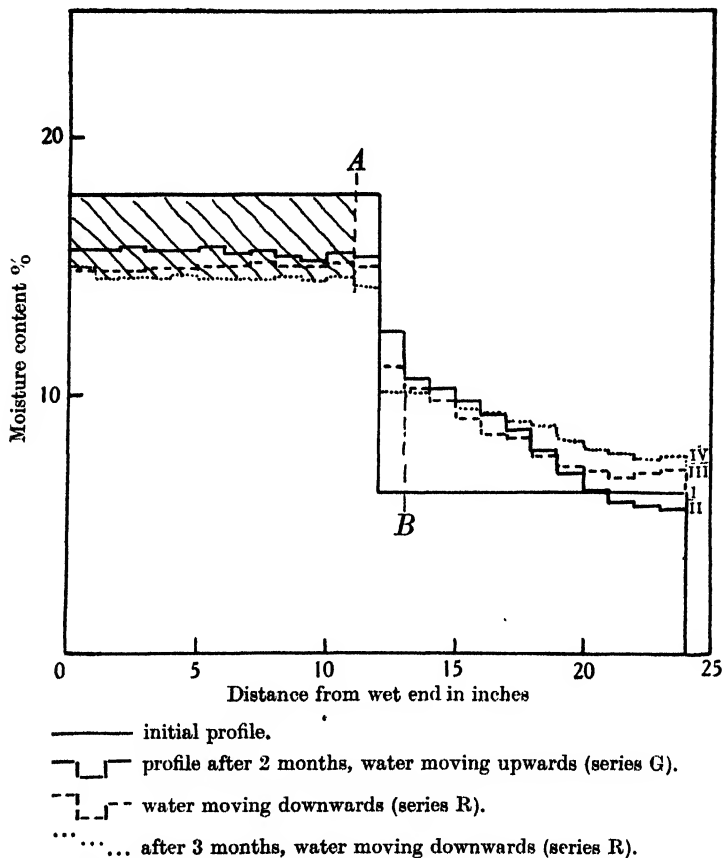


Fig. 2. Moisture profiles of series G and R of Alway & Clark's experiments.

the moisture advanced as a "step" in the manner of Fig. 1, although, as might be expected, the experimental curves showed rounded "corners" rather than sharp ones, and progressive rather than abruptly discontinuous changes of moisture content. He concluded that the liquid water was in equilibrium at the end of 123 days, and that further movement must occur only in the vapour state. It is difficult to find any justification for this statement in the published curves, since there was appre-

cial movement in the interval between the 89th and the 123rd day. He is of the opinion, nevertheless, that the column tends to uniform moisture distribution, as the diffusion theory in its simplest form requires.

According to Alway & Clark the "step" remains stationary at its initial depth but decreases in height, moisture passing from the wet side

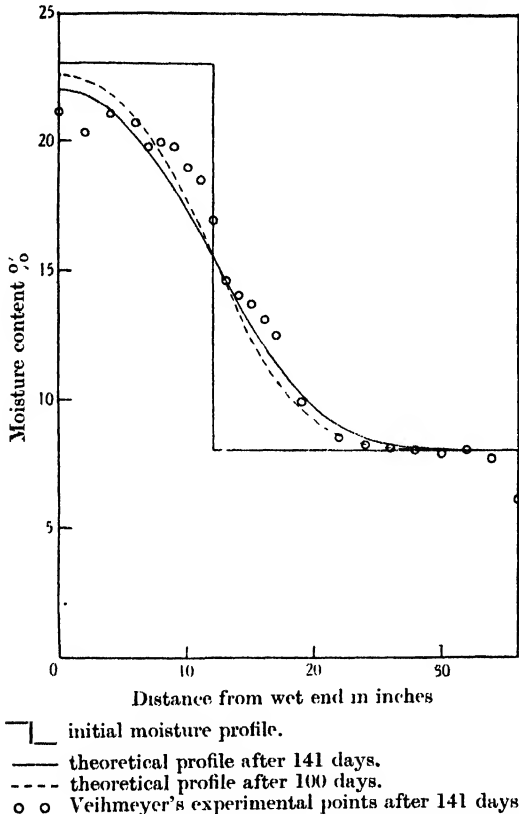


Fig. 3. Comparison of theory with Veihmeyer's experiment.

to the dry. Fig. 2 shows some of the most reliable of their results. In a large number of their experiments the average moisture content depends on the date of sampling, indicating either that water was finding its way in or out of the cylinders (a possibility ruled out by the fact that the total weights of the cylinders did not change) or that the sampling was in some way at fault. The curves chosen are those which, in addition to being, on account of the constancy of average moisture content, more reliable than the rest, also show the effects of upward, as against down-

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ward, moisture movement. Series G (Alway & Clark's nomenclature) shows the result of placing the dry layer above the wet, while series R has the same initial moisture distribution, with the difference that the wet layer is above the dry. Series R also shows the profile at the end of 3, as well as of 2, months.

Veihmeyer<sup>(7)</sup>, working with a block of wet soil between two blocks of dry soil in a vertical column, and using heavy soils, took greater

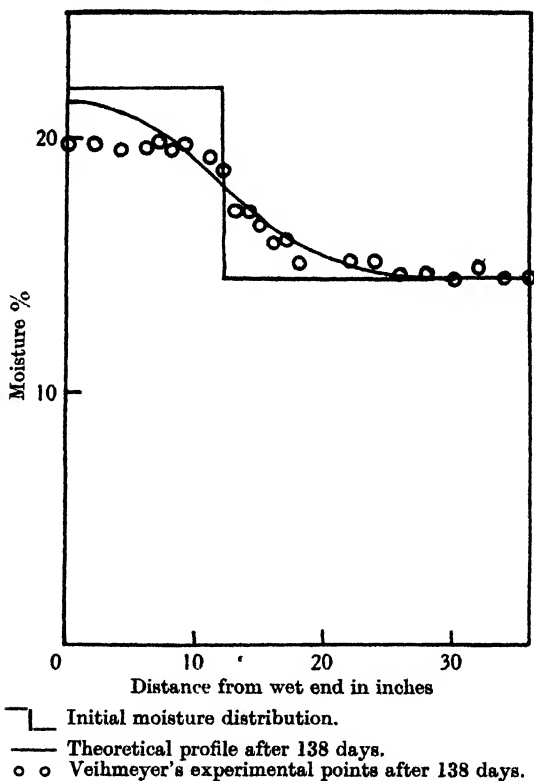


Fig. 4. Comparison of theory with Veihmeyer's experiments.

precautions to obtain good contact at the junctions, and as a result he found that the moisture contents in the separate portions were not so uniform at the end of about 4 months as was the case with Alway & Clark. There was no sudden step in the curve, but a gradient which was steepest at the junction and decreased as the distance from the junction increased. In some cases the moisture distribution in the wetter section was very nearly uniform, but such was never the case with the drier sections. Figs. 3 and 4 show some of his experimental observations.

Of the three points to be dealt with the numerical fitting of theoretical curves to observed points, as in (b), is undoubtedly the most convincing justification of the diffusion theory. With this accomplished, the qualitative explanation of those experiments which are too complicated to be amenable to mathematical treatment may be considered to rest on something more reliable than mere hypothesis.

#### EXAMINATION OF THE EXPERIMENTAL RESULTS

In endeavouring to fit theory to facts, the most satisfactory experiments are those of Veihmeyer<sup>(7)</sup> and of Alway & Clark<sup>(4)</sup>. The irrigation work of Israelsen provides boundary conditions for the diffusion equations which are not precisely stated. Shaw's results are obtained with very dry soils (initial moisture content less than 2 per cent), and as we shall see when these are discussed, it is not easy to make numerical calculations, although the main features may be accounted for. The experiments of Veihmeyer and of Alway & Clark provide initial moisture distributions which are extremely convenient for the purpose of calculation. They both began with a column of soil at one uniform moisture content placed in contact with another at a lower moisture content. Laborious graphical integration may be dispensed with and the solution of the equations obtained in analytical form. The two series of experiments are very nearly identical in form, and those of Veihmeyer were chosen for the following reasons. Firstly, the persistence of a boundary between the wet and the dry regions, together with the approximately uniform moisture distributions in each region separately, has been ascribed to the difficulty of obtaining contact between two layers of differently prepared soils as good as the contact between the various parts of any one layer. Veihmeyer recognized this difficulty, and was at great pains to reduce errors on this account. Certain of his results show that he obtained fair success (see his Fig. 42), while others show evidence of less satisfactory contact between the layers. It will be shown that the rather different results of Alway & Clark may be accounted for both qualitatively and quantitatively by this failure of contact. Secondly, Veihmeyer worked with heavy soils, for which the diffusion theory was specially introduced. Nevertheless, this theory should account for water movement in any soil in which gravity is only a minor factor, and the difference between upward and downward movement in Alway & Clark's soils (compare their series G and R) is probably sufficiently small to justify the application of the theory to their work, and in any case to those features which are not to be ascribed to gravity.

## COMPARISON OF DIFFUSION THEORY WITH VEIHMAYER'S EXPERIMENTS

The experiments concerned began in each case with a vertical column of soil built up in three equal sections, the middle one being at higher moisture content than the outer ones, and the distribution of moisture in each separate section being uniform. The moisture profiles taken some months later were very nearly symmetrical about the middle of the column, showing that the movement of moisture upwards from the wet column was effectively the same as that downward. The case is one of simple redistribution without loss by evaporation or gain by added water. Case 1 of paper I is therefore applicable, and since each half of the composite column retains its own moisture, it is convenient to deal only with one half, preferably the lower. In some cases the upper half shows an unaccountable water loss in the drier section, possibly by evaporation.

The expression giving the moisture profile  $c_t$  at time  $t$  is (equation (12) of paper I)

$$c_t = \frac{1}{l} \int_0^l c_0 dx + \sum_n \left( \frac{2}{l} e^{-\frac{k}{\sigma} \left( \frac{n\pi}{l} \right)^2 t} \int_0^l c_0 \cos \frac{n\pi x}{l} dx \right) \cos \frac{n\pi x}{l}, \quad \dots (1)$$

$$n = 1, 2, 3, 4, \dots$$

Here  $l$  is the length of the half-column under discussion,  $c_0$  is the initial moisture distribution,  $k$  is the diffusion coefficient for the soil,  $\sigma$  the weight of dry matter per unit volume of soil, and  $x$  the distance, from the wet end, at which  $c_t$  is being calculated. The simplicity is evident when we write down the expression for  $c_0$ . In Veihmeyer's experiments the initial moisture distribution was constant at  $c_1$  for a length  $l_1$  (12 in. if we take the middle of the column as our zero) and constant at  $c_2$  for the remaining distance  $l - l_1$ . Therefore

$$c_0 = c_1, \quad 0 < x < l_1,$$

$$c_0 = c_2, \quad l_1 < x < l.$$

The integrations in (1) may therefore be carried out analytically as follows:

$$\begin{aligned} \frac{1}{l} \int_0^l c_0 dx &= \frac{1}{l} [c_1 l_1 + c_2 (l - l_1)], \quad \dots (2) \\ \int_0^l c_0 \cos \frac{n\pi x}{l} dx &= \int_0^{l_1} c_1 \cos \frac{n\pi x}{l} dx + \int_{l_1}^l c_2 \cos \frac{n\pi x}{l} dx \\ &= \frac{l}{n\pi} \left[ \left( c_1 \sin \frac{n\pi x}{l} \right)_0^{l_1} + \left( c_2 \sin \frac{n\pi x}{l} \right)_{l_1}^l \right] \\ &= \frac{l}{n\pi} (c_1 - c_2) \sin \frac{n\pi l_1}{l}. \quad \dots (3) \end{aligned}$$

Expression (2) gives the average moisture content to which the profile tends as  $t$  tends to infinity. Therefore

$$c_t = \frac{1}{l} [c_1 l_1 + c_2 (l - l_1)] + \sum_n \frac{2}{n\pi} (c_1 - c_2) e^{-\frac{k}{\sigma} \left(\frac{n\pi}{l}\right)^2 t} \sin \frac{n\pi l_1}{l} \cos \frac{n\pi x}{l}. \quad \dots(4)$$

We shall select for our purpose Veihmeyer's Fig. 42, since we do not wish to risk the possibility of the experimental complications, discussed later, caused by starting with very dry soil on one side of the boundary or by the contact difficulty between soils of very different moisture content. Throughout the experiments the lengths involved are as follows:

$$l = 36'' = 91.5 \text{ cm.},$$

$$l_1 = 12'' = 30.5 \text{ cm.},$$

$$\frac{l_1}{l} = \frac{1}{3}.$$

The special conditions attaching to the selected experiments are

$$c_1 = 23 \text{ per cent (expressed as percentage of dry matter),}$$

$$c_2 = 8 \text{ per cent,}$$

$$t = 141 \text{ days.}$$

There is no direct means of calculating  $\kappa/\sigma$  in this case owing to lack of data, as was remarked in paper I, but by a process of trial and error about the probable value, the final choice was

$$\kappa/\sigma = 1 \text{ (in the C.G.D. system).}$$

This compares with a figure of 2 for the gault clay on the Cambridge University Farm, but it must be remembered that this was measured *in situ* in an undisturbed soil, while the soil in Veihmeyer's experiments was dried, then moistened to the required value, and then packed into containers, all processes which might be expected to decrease the coefficient of diffusion. The final form of the moisture profile is, by substitution of the special values in equation (4),

$$c_t = 13 + \sum_n \left(\frac{2}{n\pi}\right) \left(15e^{-141\left(\frac{n\pi}{91.5}\right)^2}\right) \sin \frac{n\pi}{3} \cos \frac{n\pi x}{l}$$

The result is shown as the full line curve in Fig. 3, Veihmeyer's experimental points being plotted on the same figure. The dotted line curve shows the theoretical distribution after only 100 days, found by substituting  $t=100$  instead of  $t=141$  in equation (5). This diagram is very instructive. It shows that the theoretical curve is in very fair agreement



with the experimental points, and also that, in the considerable interval of 41 days between the earlier and later curves, very little movement of moisture is to be expected, less possibly than the experimental errors might account for. This may be held to explain the general impression that at the end of an arbitrary period of a few months moisture movement ceases. Alway & Clark, for instance, show that movement in the third month is slight, and they average the moisture profiles at the beginning and end of this period to give a curve which is assumed to be final.

Fig. 4 shows the results of carrying out the calculations for the case of Veihmeyer's Fig. 44. The agreement is fair but not so good at the end of maximum moisture content. In fact, the chief discrepancy in all the curves is in this region, which, as we shall see, is a natural consequence of imperfect junction of the wet and dry regions. The experimental points of Fig. 4, however, indicate that the water leaving the wet section was in excess of that entering the dry section by about 40 per cent, so that a large experimental error may account for the depression of the curve at the wet end. This excessive uniformity of distribution is even more fundamentally at variance with the hysteresis theory, as we shall show in the following section.

The tendency for the moisture contents to be more uniform than the theory indicates is more marked in the experiments of Alway & Clark, who find more uniform distribution in the dry layer as well as in the wet, with a discontinuity at the junction. To this extent the two series of experiments provide conflicting evidence. It is reasonable to suppose that if, in cases where precautions were taken to secure continuity of movement at the junction, the diffusion theory accounts amply for the observed facts, then one is justified in modifying the theory by taking into account poorness of contact in order to account for the more complicated curves of Alway & Clark. The necessity of accounting for them by the diffusion theory is all the more obvious when we realize that the hysteresis theory is not a theory of water movement at all but a theory of the final state. There is, as we have seen, no evidence that the profiles described *are* final states. If the hysteresis theory were soundly based, the diffusion theory would have to be modified so that the calculated moisture profiles tended towards the non-uniform equilibrium profiles as time went on. We shall show that such modification is not only an unnecessary complication, as was pointed out in the original discussion (8) and demonstrated by the calculation of Fig. 3 of this paper, but that the hysteresis theory leads to conclusions which are at variance with the observed facts as reported by the American workers quoted.

## DISCUSSION OF THE HYSTERESIS THEORY

The main features of this theory may be seen from Fig. 5, which indicates the degree to which water is extracted from soil by various suction forces (or pressure deficiencies) and the degree to which it is remoistened as the suction force is reduced. It must be understood that the moisture contents are measured after some arbitrary interval of time subsequent to the application of the suction force, at the end of which

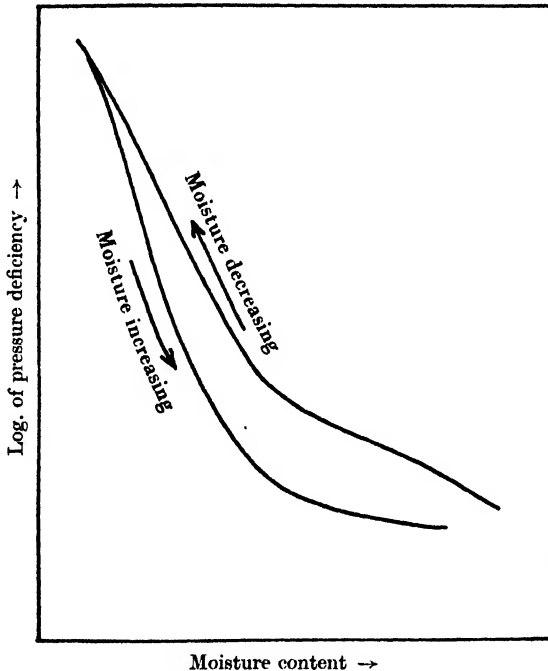


Fig. 5. Sketch of soil moisture *v.* pressure deficiency hysteresis curve.

water movement is assumed to have ceased. The suction force varies much more rapidly with moisture content in dry soil than in the wet, so following Schofield, the well-known device of plotting the rapidly varying quantity (in this case pressure deficiency) on a logarithmic scale is adopted in Fig. 5, since this, as he points out, results in a more convenient diagram. We see from this that, reduced to the simplest language, a soil which is drying requires greater suction to maintain a certain moisture content than one which is moistening. If we take the mean of the two pressure deficiencies as our zero of reference we may say that

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a wet soil is reluctant to part with its moisture and a dry one is reluctant to accept more. In consequence a dry soil is very reluctant to accept moisture at the expense of a wetter one placed in contact with it. This is held to account for the apparent fact that moisture movement ceases when there is still a difference of moisture content on the two sides of the junction, the suction forces being equal in spite of this difference.

Let us, however, transfer our attention from the very small fraction of the whole column forming the junction, which is the one part of the column that we cannot be very certain about, and consider water movements in the whole. In what way does moisture travel from the wet region to the dry? There is no agency at work selecting water uniformly from all parts of the former and distributing it uniformly to all parts of the latter. What happens is that if we imagine the soil divided into thin layers parallel to the junction surface, then the layer of dry soil on one side of the junction draws water from the thin layer of wet soil adjacent to it on the other side of the junction. The wet layer is thus left drier than before, but the loss due to water going out on one side is partly compensated for by water entering on the other side from the next thin layer of wet soil. If we accept Schofield's theory it need not necessarily be wholly compensated for in any reasonable time, since it is a case of a layer of wet soil losing moisture to a layer of drier soil. This process is continued throughout the soil, and a corresponding one takes place on the drier side, part of the gain of moisture by entrance on one side of each thin layer being compensated for by exit on the other into the adjacent drier layer. As a result a moisture gradient is built up, the limiting gradient being that which corresponds to uniform suction pressure, and consequently to zero movement of water throughout the column. We note that, far from it being possible for water to move with a perfectly uniform moisture distribution, it is necessary for the gradient to be built up to some definite lower limit before any will move at all. Compare this logical conclusion with the experiments of Alway & Clark, illustrated in Fig. 2 and quoted as the most favourable evidence for the theory. The shaded area represents the very considerable amount of moisture, in fact practically the whole amount lost by the wet soil, which passed the section at *A*, in spite of the fact that the gradient is apparently at all times zero, while a similar area from *B* to the end represents practically the whole gain of the dry soil, and this passed *B* with a moisture gradient which, if not zero, is negligibly small compared with the steep region in the small interval from *A* to *B*. Practically the whole curve, from 0 to *A* and from *B* to the end, shows varying degrees of

moisture movement with the same zero slope. For the theory to be tenable the experiments should give the predicted distribution as in Fig. 6 or a slight modification of it. It is impossible to predict how steep the final gradient in Fig. 6 should be because the hysteresis theory is not concerned with the thickness of the layers; however thin they may be they can still possess moisture contents at equilibrium differing from those of their neighbours. There is no reason why the final gradient

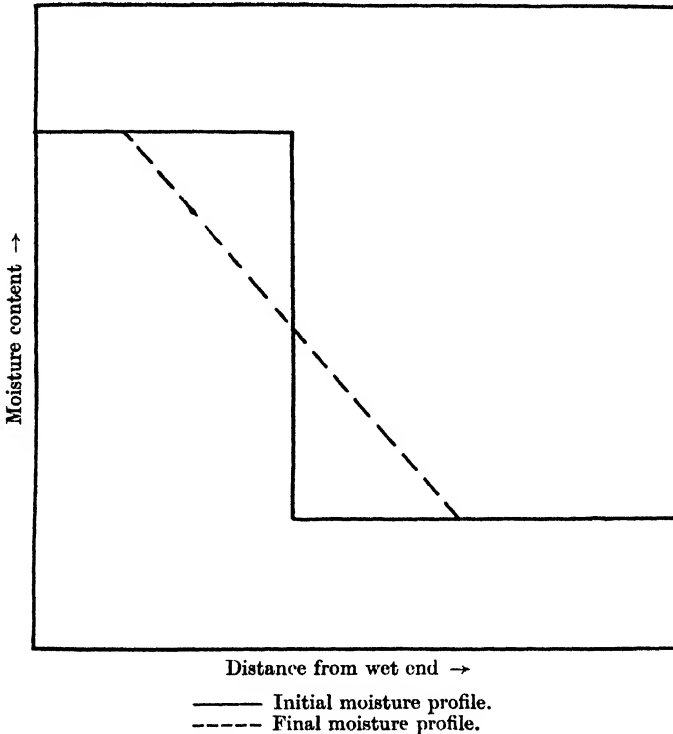


Fig. 6. Type of moisture profiles to be expected on Schofield's theory.

should not be infinitely steep, i.e. the final distribution might be identical with the initial, indicating an entire absence of water movement no matter how great the difference of moisture content may be. One can see this more easily by considering a composite column consisting of the dry section, the wet section, and between them a number of thin layers the moisture contents in which are graded up between the two extremes. The difference between the moisture contents of adjacent layers is made such that water movement from one to the other is just prohibited. The result is a moisture profile in the form of a staircase. If now the thickness

of the layers (i.e. the width of each step of the "staircase") be reduced to very small values, the "staircase" is so steep as to be unresolved by the available crude methods of moisture sampling, and the result is an increase of moisture content at the junction from the minimum to the maximum in one "step". The water is, however, still in equilibrium according to the hysteresis theory, irrespective of the extreme moisture difference. In practice the moisture content at the junction must be so graded, however sharply, between the two extremes soon after the two soils are placed in contact (in fact the experiments quoted show this). Hence the theory forbids water movement in any practical circumstances. This is because it is stated in terms of finite differences of moisture and of pressure. If it were stated in the language of the differential calculus (moisture and pressure gradients) it would be somewhat as follows. There is (for each moisture content) some moisture gradient, greater than zero, corresponding to a zero pressure gradient and consequently to absence of further water movement. The "final" profile would then be more like Fig. 6, but still the possible length of the gradient would be unlimited, or would end only at such saturation moisture contents that the wetting and drying curves coincided. This restatement also avoids an apparent paradox. If we consider a cross-section of a soil column, across which water is moving, it is losing water to the adjacent drier layer and should therefore be considered to have the pressure deficiency corresponding to the drying condition, but it is accepting water from the wetter layer on the other side and should therefore, for the same moisture content, be considered as having the lower "wetting" pressure deficiency. It clearly cannot have both at the same time. The statement of the theory in terms of pressure gradients does not involve this paradox, and is in accordance with Schofield's view<sup>(2)</sup> that the "hysteresis" is due to "microplastic" movements rather than to surface tension effects. The modification, however, merely results in the consequences of the theory being less startlingly, but still fundamentally, at variance with the experimental facts; the movements indicated in Fig. 2 are still denied.

The experiments of Shaw are equally inexplicable by the theory. Fig. 1 shows that, as the water front advances, the initially dry soil is wetted up to the full capacity of which the available water is capable, in contradiction to the hysteresis theory, and then proceeds to lose water, being now part of the wet column, in moistening more dry soil; the dry soil is moistened either to the full capacity or not at all.

We are bound to conclude that the anomalies found at the junction

are to be interpreted by reference to imperfections of contact and need not be due to complicated physical hypotheses. Such imperfections may develop in the course of the experiment in spite of the most detailed precautions, particularly when the initial difference of moisture content is large and one soil is very dry. This is due to differential shrinkage effects, as shown in Fig. 7. This is a sketch following Haines's<sup>(10)</sup> results

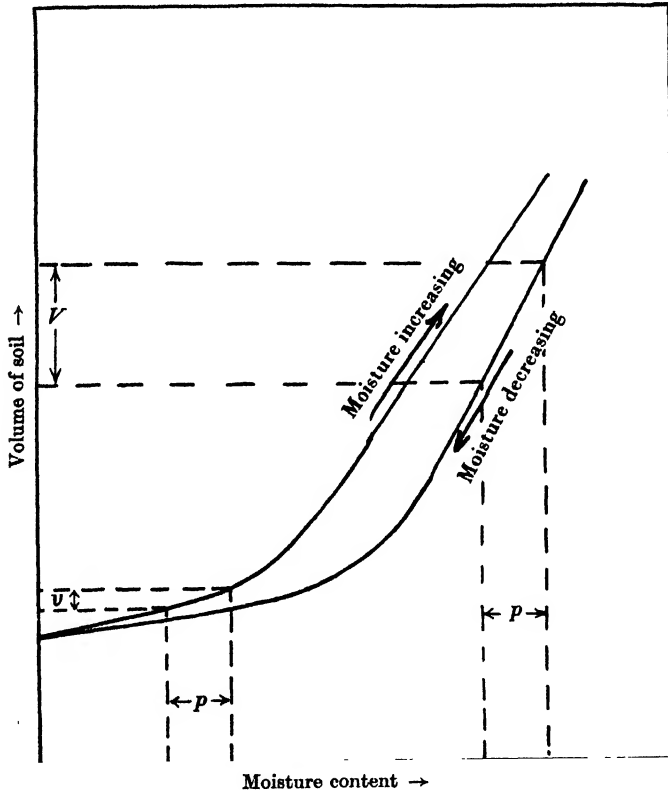


Fig. 7. Sketch of curve of soil shrinkage phenomena, after Haines.

and shows that the wet soil shrinks by a volume  $V$  upon losing a certain quantity of water  $p$ , but the dry soil, on gaining this volume  $p$ , swells only by the volume  $v$ . The volume difference is most probably located at the junction, where there is almost certainly a plane of weakness, and in any case even an invisible fracture would suffice to impede water movement. With this complication in mind we may now proceed to account for all the experiments quoted on a basis of the diffusion theory,

and to show that conflicting results are due to varying conditions of experiment.

#### DISCUSSION OF THE EXPERIMENTAL EVIDENCE IN THE LIGHT OF DIFFUSION THEORY

The experiments of Alway & Clark, and those of Veihmeyer which do not provide a very good numerical fit to the theory, may be taken together, since they are cases where the dry soil was initially at a moisture content that was so low as to be in the neighbourhood of the hygroscopic coefficient, and usually the initial difference of moisture content was large. If it be assumed that contact throughout the experiment was poor, this condition could be approximated to theoretically by considering moisture movement during successive periods of alternate perfect contact and perfect isolation. If it be considered that complete isolation occurred at some time during the experiment, this case also could be worked out numerically. In the absence of precise information concerning the periods of good and bad contact one would be forced to employ a process of trial and error. By choosing suitable intervals of time and suitable values of  $\kappa/\sigma$  almost any probable final distribution could be accounted for with reasonable accuracy, so that the process is laborious without being very profitable. It may be seen broadly, however, that in the periods of contact water would move across the junction according to diffusion laws, tending towards uniform distribution throughout, but that in periods of isolation the water which had so moved would tend to redistribute itself, tending to uniform but different moisture distribution in each section separately (see Fig. 8). Additional evidence for this is provided by Fig. 2. Here, curve IV, obtained 1 month later than curve III, shows signs of redistribution of moisture in the dry region, tending towards uniformity, but complicated by addition of water from the wet section. The evidence is not very good since, from the nature of the earlier curve with its small gradients, redistribution could be but slow.

Again, if we examine Alway & Clark's curves of their series T we find that an apparently final state shows a moisture content of 20 per cent on the wet side and on the dry side a content beginning at 13.5 per cent near the junction and approaching 11.5 per cent farther away. Yet Veihmeyer, beginning one of his experiments at approximately this "final" distribution (see Fig. 4) and working with heavy soil in which movement might be expected to be much slower, finds that considerable movement takes place. It is only fair to assume that the

cessation of moisture movement noted by Alway & Clark was due to development of poor contact, and that, had they remedied this state of affairs, the movement would have continued.

The experiments of Shaw(6) must be accounted for somewhat differently, since there was no line of demarcation between differently prepared soils. A head of water was applied at one end and the water

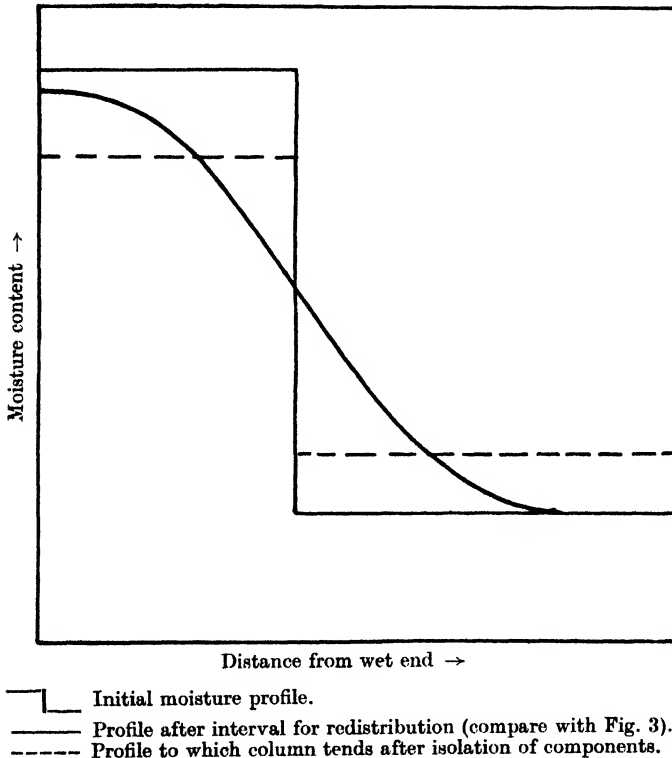


Fig. 8. Theoretical curves accounting for the observations of Alway & Clark.

front was found to advance as in Fig. 1, modified, naturally, by smoothing of the corners. This is quite reasonable when we consider that the soil was dried initially to a moisture content of less than 2 per cent of the dry weight. There can have been no continuity of moisture film. The wet soil must, as it were, have been deprived of its intelligence service; it could not have known the length of the column into which it was expected to diffuse. With circumstances such as these the effective length of the column,  $l$  of the theory of paper I, was a variable quantity. It was



always a little greater than the length of the wetted column, the end always being located a little in advance of the water front as the latter moved along, quite irrespective of the quantity of dry soil beyond it.

Reference to the mathematical details will show that such a boundary condition renders exact numerical work out of the question, but once again the diffusion theory accounts for the general behaviour. A rough approximation, rendering calculation in stages possible, would be to consider a length  $l$  a little greater than that of the wetted column, and, with a suitable value of  $\kappa/\sigma$  (by trial and error in the absence of data) allow the moisture sufficient time to redistribute itself so that the content at the end ( $x=l$ ) reached a certain predecided minimum value. An increment would then be added to  $l$  and the process repeated. The choice of the minimum water content which must be reached before the movement could continue beyond the end of the effective column, and of the amount of each successive increment of length, are too arbitrary to make calculations of value, but it is easy to see that a suitable choice would enable a perfect advancing "step" to be demonstrated, of any steepness desired. By remaining always a little ahead, the effective end of the column draws on the water front in much the same way as the time-honoured carrot draws on the donkey.

Enough has been done to show that the diffusion theory is not only adequate to account for known water movements of the type determined by surface-tension forces (as distinct from gravitational movements), but also that it is, so far, alone in providing fair numerical fit to those experiments the conditions of which are known sufficiently well to allow any theory at all to be applied. It is to be hoped that it will eventually be superseded by one giving a picture of the mechanism involved in water movement in soils, but such a theory must provide an agreement with practical experience at least as good, both qualitatively and quantitatively, as that yielded by the diffusion theory. Owing to the extreme complexity of the soil considered as a water-conducting medium we may have to wait some time for such a physical theory.

Further experiments recently carried out with the gault clay soil of the Cambridge University Farm show that, for field conditions, the simple theory must in certain respects be modified in accordance with the tentative suggestions at the conclusion of paper II, and further experiments are in progress to this end.

## SUMMARY

It is shown that the theory of diffusion of water through soil accomplishes three things:

- (a) It accounts qualitatively for the water movement from wet to dry soil observed by the American workers quoted.
- (b) It fits the best experiments numerically.
- (c) It is at present alone in doing this.

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# THE NUTRITION OF THE BACON PIG

## I. THE INFLUENCE OF HIGH LEVELS OF PROTEIN INTAKE ON GROWTH, CONFORMATION AND QUALITY IN THE BACON PIG

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(With Plate IV)

### INTRODUCTION

THE present practice of basing payment for bacon pigs not merely on carcass weight, but also on carcass quality as expressed in the factory grading results, has led necessarily to an enlargement of outlook on the part of the investigator in the domain of swine nutrition. The interest of the experimenter in the past has tended to be restricted to such questions as the influence of nutritional factors on the rate of live-weight increase and the efficiency of food conversion, the problem of how feeding may influence carcass quality and conformation not having received the attention its importance merited.

While the questions of the rate of growth and the efficiency, in terms of meal consumption, with which such growth is achieved are still matters of primary economic importance, it is realized that much more attention must be devoted than heretofore to the study of carcass quality and conformation in relation to feeding. In present circumstances, a nutritional investigation can scarcely be considered complete unless a substantial part of the research is carried out in the bacon factory following the slaughter of the experimental animals. The general problem is one of elucidating the extent to which the factor of nutrition, as opposed to the factors of breed and strain, may influence quality and conformation. That the nature of the ration may influence profoundly the rate of live-weight increase and the efficiency with which the food

is converted into live-weight increase is now well known; can it also influence to any significant extent the conformation of the pig and the quality of the resultant carcass?

The particular phase of the general problem selected for study in the present investigation is the influence of feeding rations containing much higher amounts of protein than are at present considered necessary from the standpoint of optimum rate of growth. An impressive amount of research has been done in past years in which the influence on the rate of live-weight increase of feeding rations of relatively low-protein content has been studied. It is by means of such valuable work that the minimum protein requirements for quick growth in pigs have been established. The other aspect of the problem, namely, the possible effect on the bacon carcass of feeding rations of relatively high-protein content, has received but scant attention. Indeed, it required the present demand for leaner carcasses to call forth curiosity on this aspect of the problem, since manifestly economy in feeding is scarcely to be looked for in the more generous use of protein-rich foods in the pig's ration. The present investigation, therefore, is not directly concerned with the economics of the production of bacon pigs, but with an attempt to secure a fuller understanding of the factors that may determine the production of lean, rather than fat, in the body of the animal.

The investigation has a further object, however; namely, to find out whether the ordinary group-feeding method of conducting trials leads to the same broad conclusions as may be drawn from the more precise, but much more troublesome and laborious method of individual feeding. With the facilities at our disposal, it is now possible to study any given problem side-by-side by the two methods of experimentation. The opportunity is thus afforded of estimating what degree of reliability is to be attached to results that have been obtained by the much-criticized group-feeding method, where the individual consumption of the animals under experiment cannot be ascertained. In this phase of the work the experimenters have had the valuable co-operation of their colleague, Dr J. Wishart, who is responsible for the whole of the statistical examination of the data as well as for the writing of the statistical section.

#### THE EXPERIMENTAL PIGGERY

Before the commencement of the present trial, extensive structural alterations were carried out in the experimental piggery (Danish type) with a view to rendering the accommodation suitable for the purposes of the investigation. One side of the piggery was left unaltered. It consists

of three equal pens, with dunging passages, each pen having accommodation for ten pigs. On this side, therefore, it is possible to undertake the comparison of the influence of three feeding treatments by the method of group feeding.

The other side of the piggery was entirely redesigned to meet the requirements of the individual-feeding technique. This side of the house is now divided by wooden partitions into five equal pens, with the usual dunging passages. Each pen accommodates six pigs, which, when not feeding, live together as a group. At feeding times, however, each of the pigs is able to pass through a sliding gate into its own small feeding compartment (4 ft. 11 in. by 1 ft. 6 in.) and consume its ration undisturbed by the other pigs in the group. The feeding arrangements will be comprehended by reference to the accompanying photographs (Pl. IV, figs. 1 and 2). The dimensions of the feeding compartments are such as to enable a 200-lb. pig to be accommodated in comfort. The partitions consist of frameworks of painted hollow iron bars,  $\frac{3}{8}$  in. diameter and  $3\frac{1}{2}$  in. apart. Should it be desired, the whole system of iron frameworks can be dismantled without difficulty and the pens used for conducting trials by the group-feeding technique. The vertically sliding gates which afford ingress to the feeding compartments can be operated easily from the central passage by means of wire ropes passing over pulleys and suitably weighted at the free ends.

#### THE FEEDING TREATMENTS

The three feeding treatments designed to throw light on the influence of high protein feeding on quality and conformation are summarized in Table I.

*Treatment A.* This represented a normal type of feeding for pigs intended for bacon. The ration of the weaners contained 12 per cent of white fish meal. This was reduced to 10 per cent at 90 lb. live weight (L.W.), while at 150 lb. L.W. the fish meal was omitted altogether, the protein-rich food at this stage consisting of 5 per cent of a mixture of equal parts of meat meal and ex. soya-bean meal.

*Treatment B.* 10 per cent of the barley in the weaners' ration was in this case replaced by 10 per cent of a mixture of equal parts of meat meal and ex. soya-bean meal, making, with the fish meal, 22 per cent of protein-rich food in all. By the time the final period of feeding had been reached, the amount of protein-rich food had been reduced to 15 per cent of the total ration and was now composed of equal parts of the meat meal and ex. soya-bean meal.



Fig. 1. Showing individual-feeding pens (by courtesy of *Farmer's Weekly*).



Fig. 2. Showing individual-feeding arrangements (by courtesy of *Farmer's Weekly*).



Table I. *Summary of feeding treatments*

	Treatment A (parts by weight)	Treatment B (parts by weight)	Treatment C (parts by weight)
<i>Up to 90 lb. L.W.:</i>			
Barley meal	55	45	35
Weatings	31	31	31
Lucerne meal	2	2	2
White fish meal	12	12	12
Meat meal	—	5	10
Ex. soya-bean meal	—	5	10
Percentage crude protein in ration	17.48	22.13	26.77
<i>90–150 lb. L.W.:</i>			
Barley meal	65	55	45
Weatings	23	23	23
Lucerne meal	2	2	2
White fish meal	10	10	10
Meat meal	—	5	10
Ex. soya-bean meal	—	5	10
Percentage crude protein in ration	15.93	20.58	25.22
<i>150 lb. L.W. to slaughter:</i>			
Barley meal	80	70	60
Weatings	13	13	13
Lucerne meal	2	2	2
White fish meal	—	—	—
Meat meal	2½	7½	12½
Ex. soya-bean meal	2½	7½	12½
Minerals*	1	½	—
Percentage crude protein in ration	12.25	16.94	21.66

\* Composed of 1 part by weight of common salt to 3 parts of ground chalk.

*Treatment C.* Under this treatment as much as 20 per cent of the barley meal in the ration of the weaners was replaced by the mixture of meat meal and ex. soya-bean meal, making, with the fish meal, the high total of 32 per cent of protein-rich food. Even in the final period of feeding, the amount of protein-rich food, consisting at this stage of equal amounts of meat meal and ex. soya-bean meal, had not fallen below the high level of 25 per cent of the ration. It was feared that such high allowances of protein-rich food, designed to emphasize the effects, if any, of high-protein feeding, might react adversely on the health of the animals. That pigs, however, in the early stages of growth, when presumably they have the maximum capacity for transforming the protein of food into flesh protein, are able to thrive on rations extremely rich in protein may be inferred from the New Zealand practice of rearing porkers on a diet composed substantially of separated milk and meat meal. It may further be noted that the sow's milk, on which the young pigs mainly subsist up to weaning, contains, on the basis of dry matter, as much as 46 per cent of protein. In the light of these facts it could scarcely be considered unduly venturesome to introduce the weaners



on treatment C to an initial ration containing 26.77 per cent of protein, and indeed, as will be shown later, all fears respecting the health of the pigs on the high-protein rations proved groundless.

The rations in every case contained 2 per cent of English lucerne meal. It was assumed that the vitamin potency and iron content of this component of the ration would obviate the necessity of supplying fresh green food to the pigs during the trial, and judging from the excellent health maintained by the animals throughout the whole experiment it would appear that this assumption has been justified. All the pigs were given access once every week to a small allowance of coal dust.

The composition of the feeding stuffs, based on their average moisture content throughout the trial, is shown in Table II. An attempt had been made during the preparatory stages of the work to secure a true meat meal displaying both low oil and low ash content. This attempt was only partly successful, however, since although the oil content of the meat meal was only 5.0 per cent, the amount of ash was relatively high at 15.3 per cent. The data for the lucerne meal suggest that this product had been made from a crop in a very early stage of growth. The green colour had been well preserved in the artificial-drying procedure.

Table II. *Average composition of feeding stuffs*

	Barley meal	Weatings	Lucerne meal	White fish meal	Meat meal	Ex. soya- bean meal
	%	%	%	%	%	%
Moisture	16.2	14.9	10.5	16.3	10.0	14.4
Crude protein	8.9	15.3	24.8	61.2	66.2	44.5
Ether extract	2.1	4.2	6.2	4.9	5.0	1.5
N-free extractives	66.7	58.4	33.0	—	3.5	29.4
Crude fibre	4.0	4.4	15.7	—	—	5.0
Ash	2.1	2.8	9.8	17.6	15.3*	5.2
Iodine value of oil†	118.3	126.0	121.8	151.0	52.6	104.9

\* Including 4.2 per cent chloride expressed as NaCl.

† As determined on petrol-ether extract.

The size of the ration fed to the pigs was adjusted week by week to conform with the live weights ascertained at the weekly weighing of the animals. The data in Table III show how the meal supply was scaled in relation to live weight. The figures in the food column represent weights of meal that have been found by experience to be a reasonable measure of appetite in pigs at different live weights. In the case of the group feeding, the amount of meal was determined by adding together the separate allowances, based on live weight, of the individuals in the group. The meal throughout was fed as a fairly thick slop, and fresh water was available at all times in all the pens.

Table III. *Feeding chart\**

L.W. in lb.	lb. meal	L.W. in lb.	lb. meal
20	1.10	120	5.30
40	2.10	140	5.90
60	3.00	160	6.45
80	4.00	180	6.70
100	4.60	200	7.00

\* Changes in meal allowance shown for live-weight increments of 20-lb. Adjustments to be made for intermediate live weights.

At this stage it is convenient to point out that with the individually fed pigs, it is possible to change over from one type of ration to another at the exact live weights at which such changes should be made. In the case of the group-fed pigs, however, these changes must be made when the *average* weight of the pigs in the group has reached the required value. It follows that some of the animals are changed over before they have attained the required live weight, whereas the more thriving pigs in the group remain on the old ration for some time after reaching the live weight at which the change-over should have been made. Herein, therefore, lies one of the advantages, from the experimenters' point of view, of the individual lay-out. Nevertheless, since the pig-feeder must necessarily ration his animals as groups, it is essential, in order to satisfy the practical man, to discover whether conclusions drawn from individual-feeding trials are capable of substantiation by group-feeding trials. It is partly with this in mind that both experimental techniques are being used in this series of investigations.

#### THE EXPERIMENTAL ANIMALS

The pigs were all pure-bred Large Whites, bred by Mr W. S. Mansfield, M.A., the Director of the University Farm. During the 10 days before farrowing, each sow had been harnessed to a 50-ft. length of chain on pasture, access being given to the shelter of a small hut. The sows were taken off the chain a few hours before farrowing, this freedom being retained until 24 hours after farrowing. They were then harnessed to their chains again until the date of weaning the litters. Each sow had its own hut, the site of this on the pasture being changed every seventh day. The young pigs were allowed to roam at will, and at 28 days of age were given a suitably balanced supplement. Both sows and litters were dry-fed during the suckling period, but an abundant supply of drinking water was always available.

On the morning of 22 January, 1935, sixty-two pigs were delivered

at the experimental piggery. They came straight from the sows on grass. Of the eight sows, of which these pigs were the progeny, seven had farrowed between 25 and 28 November, while one (sow 175) had farrowed on 18 November. None of the litter of this last-named sow was included in the lay-out of the individually-fed pigs.

The pigs were weighed on arrival, and for the first week were allowed to become accustomed to the indoor conditions. During this time they were all fed on ration A containing 12 per cent of fish meal. On account of the outdoor method of rearing on fresh pasture, it was considered unnecessary to "worm" the young pigs. They were again weighed, before feeding, on the morning of 28 January, and on the basis of these and the previous week's weight data were grouped for experiment and brought on to their respective feeding treatments.

#### THE EXPERIMENTAL LAY-OUT

*Individual-feeding lay-out.* The simplest method of comparing the effects of three feeding treatments would be to use three pigs of the same sex, having as nearly as possible the same weaning live weight, and belonging to the same litter. Each of the five pens in the individual feeding lay-out contained six pigs from a given litter, three hogs and three gilts. The three feeding treatments, A, B and C, were applied to the three hogs and also to the three gilts, so that each pen provided two simple comparisons in which sex was taken into account. In all, therefore, the lay-out permitted of ten separate comparisons.

As will be seen from Table IV, in which the precise details of the lay-out are shown, each of the five litters was deliberately divided into three heavy hogs (or gilts, as the case may be) and three light gilts (or hogs). The positions of the "heavies" and the "lights" were randomized within the pens, as were also the applications of the three feeding treatments within the "heavies" and the "lights". Only in pen IV is there any irregularity in the distribution of the pigs among the feeding compartments. This arose from considerations of weaning live weights, but it was shown later that this precaution respecting feeding positions in this pen was superfluous. Indeed, as will be made clear in the statistical section, it is unnecessary to adopt the procedure of dividing the litters into "heavies" and "lights". The three gilts (or hogs) constituting a single comparison may quite well be selected by random procedure without reference to live weight and the results of the comparison corrected for difference in initial live weight.

*Group-feeding lay-out.* The animals remaining after designing the

Table IV. *Lay-out of experiment*

Individual-feeding lay-out					Group-feeding lay-out			
	No. of pig	Sex	Treat-ment	Weaning L.W. (lb.)	No. of pig	Litter	Sex	Weaning L.W. (lb.)
Pen I (litter of sow 357)*:					Group I (feeding treatment A):			
H†	993	Gilt	A	36	937	175	Hog	33
	989	Gilt	B	36	948	437	Hog	34
	987	Gilt	C	35	958	436	Hog	33
L†	994	Hog	C	34½	962	436	Hog	30½
	991	Hog	B	28½	953	437	Hog	21
	988	Hog	A	28½	972	456	Gilt	46½
Pen II (litter of sow 327)*:					941	175	Gilt	42½
L	1010	Gilt	B	23	959	436	Gilt	28½
	1003	Gilt	C	20	969	208	Hog	37½
	1004	Gilt	A	25½	992	357	Gilt	34
H	1007	Hog	C	26½	Total weaning L.W. 340½			
	1013	Hog	A	27	Group II (feeding treatment B):			
	1008	Hog	B	27	940	175	Hog	46½
Pen III (litter of sow 342)*:					943	437	Hog	26½
L	995	Gilt	C	26	960	436	Hog	34½
	997	Gilt	A	26	955	436	Hog	30
	998	Gilt	B	30½	954	437	Gilt	31½
H	996	Hog	B	31½	971	456	Hog	43½
	1001	Hog	C	31	965	208	Gilt	30½
	999	Hog	A	30½	986	357	Gilt	38
Pen IV (litter of sow 208)†:					1009	327	Gilt	27½
H	963	Gilt	C	39	1000	342	Hog	27
	970	Hog	A	35	Total weaning L.W. 335½			
	967	Gilt	B	38	Group III (feeding treatment C):			
L	968	Gilt	A	33	942	175	Hog	47
	966	Hog	B	29½	952	437	Hog	22½
	964	Hog	C	30½	956	436	Hog	35½
Pen V (litter of sow 437)†:					957	436	Hog	38
L	946	Gilt	B	28	944	437	Gilt	28
	947	Gilt	A	24	973	456	Hog	28
	950	Gilt	C	23½	938	175	Gilt	41
H	945	Hog	B	28½	961	436	Gilt	29½
	951	Hog	C	29	990	357	Hog	39½
	949	Hog	A	33	1005	327	Gilt	28½
					Total weaning L.W. 337½			

\* Sire: "Moulton".

† H="heavies"; L="lights".

‡ Sire: "Davidson".

lay-out for the individually-fed pigs were utilized in the formation of three groups of ten for the purpose of comparing the effects of the three feeding treatments by the method of group feeding. Among a number of conditions that should be observed in such trials, it is desirable that each group should contain the same number of animals from any given litter and an equal number of animals of the same sex; further, the combined live weights of the animals in each group should be as nearly equal as possible.

It was unfortunately not possible to realize these conditions in their entirety. The experimenter, when making such groupings, is naturally limited by the nature of the experimental material at his disposal. How closely the actual groupings approximated to an ideal lay-out will be seen by referring to the details shown in Table IV. When comparing the results obtained by the two experimental techniques, it should be kept in mind that the group-feeding lay-out, because of factors beyond the experimenters' control, was slightly more open to criticism than that adopted in the individual-feeding trial. It should be stated, however, that the selection of both heavy and light pigs for the individual-feeding lay-out effectively safeguarded against any tendency to leave behind the poorer pigs for the group-feeding trial.

#### THE COURSE OF THE TRIAL

The pigs were brought on to their respective feeding treatments after the second weighing on the morning of 28 January. At the end of a further week of feeding, all the pigs were weighed again (before their morning feed) on 3 successive days (4, 5 and 6 February). The means of the three weighings were taken as representing the initial live weights of the pigs in the trial, the first day of the trial proper being 5 February. Thereafter the pigs were weighed at weekly intervals.

As frequently happens in such trials it was soon discovered that one pig (gilt 986 in group II) was outstripping all the others in respect of live-weight increase (L.W.I.). This pig attained 200 lb. L.W. during the week ending 7 May and should therefore at this date have been sent to the factory. Such a procedure, however, would have involved a discontinuance of the comparison of the effects of the feeding treatments on L.W.I. at an earlier date than was desirable. It was decided, therefore, to allow this pig to remain in the trial beyond 200 lb. L.W., and it was ultimately slaughtered at 230 lb. fasted L.W. on 30 May. The final date of the trial, from the standpoint of comparing the influence of feeding treatment on the rate of L.W.I., was 28 May." At this stage, all the pigs were weighed on three successive mornings (27; 28 and 29 May), the mean weights being taken as the final live weights in the comparison. Full records for both the individually-fed and group-fed pigs were still kept, however, until every animal had attained 200 lb. L.W.

All the pigs were fasted before slaughter, receiving their last meal on the morning of the day preceding the day of slaughter. The first batch of fifteen pigs was slaughtered on 30 May. Thereafter, batches were transported to the factory for slaughter at intervals of a week, the final

batch being slaughtered on 3 July. The pigs were sent by road in a motor lorry to the St Edmundsbury Farmers' Co-operative Bacon Factory, Elmswell, Suffolk. After the fasted live weights had been determined, they were dispatched at about 9.15 a.m., arriving at the factory at about 11.30. They were weighed again on arrival and slaughtered at about 1 p.m.

The whole trial proceeded satisfactorily from the standpoint of the health of the pigs. There were no mishaps or cases of serious disturbance in the condition of the animals, and no week during the whole experiment in which every pig did not register a gain in live weight. The animals were obviously housed in comfort; wheat straw was used liberally for litter and, in consequence of improvements effected in the piggery, the temperature was equable and the atmosphere free from surplus moisture.

A disadvantage, from the experimental standpoint, of the individual-feeding technique was manifest in the early stages of the trial. Because of the necessity of having to train the individually-fed pigs to enter their proper feeding compartments, these animals were subjected to much more "handling" during this early period than was necessary in the case of the group-fed pigs. The extra disturbance involved in this treatment had a natural reaction on the individually-fed pigs, so that they did not display, in the initial stages, the same degree of thriving condition as the group-fed animals. This state of affairs lasted only a short time, however. The individually-fed pigs soon accustomed themselves to the conditions and after about a fortnight began to require little or no direction from the worker in charge when entering their feeding compartments. From this stage onwards the total weekly gain of the pigs in the individual feeding lay-out, instead of being, as in the first 3 weeks after forming the groupings, slightly smaller than that of the group-fed pigs, began to be higher. The data relative to this behaviour of the individually-fed animals, which was probably to be attributed to their being able to feed undisturbed, will be analysed in a later section.

The pigs were kept under careful observation during the course of the trial with a view to noting any distinguishing features that might be attributed to differences in feeding treatment. It was found, for example, that the animals on the high-protein diets (B and C) drank more water than those on the normal-protein ration (A). The C pigs were the thirstiest and, judging from the condition of the bedding, these animals also urinated much more freely than the A pigs. This may have been caused by the inclusion of the meat meal, which was rather salty to the taste, in the rations of the B and C pigs (see Table I). On the

other hand, it was consistent with the possibility that much of the extra protein in the B and C diets was not being utilized for flesh production, but was leading instead, by deamination, to a higher level of urea formation and elimination.

The skins of the pigs on the high-protein diet C were notably duller than those of the other pigs in the first half of the feeding trial. The C pigs also displayed at times a tendency to develop slight rashes and small pimples. No "scouring" resulted from feeding such high allowances of protein-rich food; indeed, the tendency at times was rather in the direction of slight constipation, but at no period of the trial was this in any degree serious. With the progress of the experiment, the skins of the C pigs showed continuous improvement and generally were little inferior to those of the A pigs when slaughter weight was reached. It may further be noted that the high-protein pigs displayed throughout as keen a zest for their food as the pigs receiving the normal allowances of protein. They exhibited no symptoms that might have pointed to depression consequent on high-protein intake, but were generally as bright and active as the other pigs under experiment.

No consistent outstanding effects of feeding treatment on shape and conformation were discernible to the eye when the pigs had arrived at slaughter weight. Some 4 or 5 weeks before this live weight was reached, it was thought that the pigs on the high-protein diet C represented a leaner type of animal than the animals on the A and B diets, but these differences to the eye were almost wiped out in the last month by the manner in which the C pigs developed heaviness round the shoulders during this final period of feeding. It was evident that there was a considerable range of variation in respect of conformation among the pigs *under any one feeding treatment*, and it was felt at the time that no useful conclusions could be drawn about the relative suitability of the differently-fed pigs for bacon until actual post-slaughter measurements of the carcasses were available.

#### PRE-SLAUGHTER RESULTS

The pre-slaughter data, omitting the records of the intermediate weekly weighings of the individual pigs, are conveniently summarized in Tables V, VI, VII and VIII. Table V shows the initial live weights of the sixty pigs on 5 February and their live weights on 28 May, on which date the comparison of the influence of the feeding treatments on L.W.I. was discontinued as a consequence of the dispatch of the first consignment of pigs to the bacon factory. Details of meal consumption

Table V. *Live-weight gains and meal consumption over experimental period of 16 weeks (5 February to 28 May)*

Individually-fed pigs						Group-fed pigs					
Feeding treatment	No. of pig	Sex	L.w. on 5 Feb. lb.	L.w. on 28 May lb.	Total meal consumed lb.	Litter	No. of pig	Sex	L.w. on 5 Feb. lb.	L.w. on 28 May lb.	
Pen I (litter of sow 357):						Group I (feeding treatment A):					
A	993	Gilt	48½	204½	542.15	175	937	Hog	45½	180	
B	989	Gilt	48	205½	552.30	437	948	Hog	45½	196	
C	987	Gilt	48½	203½	549.15	436	958	Hog	44½	206	
C	994	Hog	47½	191½	536.90	436	962	Hog	41½	178	
B	991	Hog	39	173½	481.25	437	953	Hog	28	161½	
A	988	Hog	38½	189	495.25	456	972	Gilt	55	190	
Pen II (litter of sow 327):						175	941	Gilt	57½	217	
B	1010	Gilt	32½	179½	473.90	436	959	Gilt	37	187	
C	1003	Gilt	28½	166	433.40	357	969	Hog	48	200	
A	1004	Gilt	32½	182½	471.45	992	992	Gilt	47	199	
C	1007	Hog	37	173	479.85	Group II (feeding treatment B):					
A	1013	Hog	35	163½	452.85	175	940	Hog	59	203	
B	1008	Hog	38	196½	510.30	437	943	Hog	34½	154	
Pen III (litter of sow 342):						436	960	Hog	44½	157	
C	995	Gilt	33	154½	426.35	436	955	Hog	37½	172	
A	997	Gilt	35	181	476.60	437	954	Gilt	42	209½	
B	998	Gilt	41	186	496.00	456	971	Hog	54½	191	
B	996	Hog	45½	177	491.50	208	965	Gilt	41	180½	
C	1001	Hog	41½	182	500.35	357	986	Gilt	52½	235	
A	999	Hog	41	186½	493.80	327	1009	Gilt	37	170	
Pen IV (litter of sow 208):						342	1000	Hog	37	177	
C	963	Gilt	50½	213	548.00	Group III (feeding treatment C):					
A	968	Gilt	45½	213	550.30	175	942	Hog	59	201	
B	967	Gilt	46	195	512.15	437	962	Hog	32	148½	
A	970	Hog	48	210	556.95	436	956	Hog	47½	163½	
B	966	Hog	39½	180½	495.25	436	957	Hog	48	191½	
C	964	Hog	42½	195	526.75	437	944	Gilt	38	154½	
Pen V (litter of sow 437):						456	973	Hog	39	198	
B	946	Gilt	37	190½	496.65	175	938	Gilt	52½	184	
A	947	Gilt	32½	172½	453.25	436	961	Gilt	37	180½	
C	950	Gilt	30½	167½	442.05	357	990	Hog	53½	197	
B	945	Hog	39½	186½	499.80	327	1005	Gilt	39½	154½	
C	951	Hog	39½	179½	490.00						
A	949	Hog	43	207	536.55						



Table VI. *Average weekly live weights of pigs under different feeding treatments*

Date	Individually-fed pigs			Group-fed pigs		
	Treatment	Treatment	Treatment	Treatment	Treatment	Treatment
	A	B	C	A	B	C
	lb.	lb.	lb.	lb.	lb.	lb.
5 Feb.	39.9	40.6	39.9	44.9	43.9	44.6
12 "	45.5	46.0	45.4	50.7	49.3	50.2
19 "	51.4	52.2	52.2	57.2	56.4	56.7
26 "	58.8	59.1	59.2	64.1	63.4	63.2
5 Mar.	67.4	67.6	67.4	72.3	70.9	71.0
12 "	76.9	76.7	75.7	80.5	79.7	78.0
19 "	84.9	84.6	83.1	88.4	86.9	85.6
26 "	93.1	92.8	91.0	95.9	94.2	92.6
2 Apr.	102.8	102.0	100.3	105.2	103.5	100.4
9 "	113.6	111.8	109.2	115.3	113.9	110.0
16 "	124.4	122.2	119.1	125.1	122.2	117.9
23 "	135.0	132.3	129.0	135.0	132.6	127.3
30 "	147.8	143.9	140.7	145.3	143.5	137.6
7 May	158.0	153.5	150.0	155.0	153.2	145.4
14 "	168.6	164.8	160.0	167.3	164.6	157.4
21 "	179.6	175.2	170.6	175.5	174.0	166.6
28 "	190.9	187.1	182.6	187.4	184.9	177.3

Table VII. *Mean rates of L.W.I. and food conversion factors\**

Treatment	Individually-fed pigs					Group-fed pigs				
	Range of	Days	lb. meal	Mean	Mean	Range of	Days	lb. meal	Mean	Mean
	L.W.I. lb.	re- quired	consumed by 10 pigs	lb. L.W.I. per day	lb. meal per lb. L.W.I.	L.W.I. lb.	re- quired	consumed by 10 pigs	lb. L.W.I. per day	lb. meal per lb. L.W.I.
A	39.9-90	47	1391.85	1.07	2.78	44.9-90	43	1376.65	1.05	3.05
B	40.6-90	47	1391.90	1.05	2.82	43.9-90	44	1395.00	1.05	3.03
C	39.9-90	48	1425.00	1.04	2.84	44.6-90	45	1439.10	1.01	3.17
A	90-150	38	1978.65	1.58	3.30	90-150	43	2183.55	1.40	3.64
B	90-150	42	2138.10	1.43	3.56	90-150	43	2170.70	1.40	3.62
C	90-150	43	2175.35	1.40	3.63	90-150	47	2380.70	1.28	3.97
A	150-190.9	27	1658.65	1.52	4.05	150-187.4	26	1596.70	1.44	4.27
B	150-187.1	23	1479.10	1.61	3.99	150-184.9	25	1516.65	1.40	4.35
C	150-182.6	21	1332.45	1.55	4.09	150-177.3	20	1191.85	1.37	4.36
A	39.9-190.9	112	5029.15	1.35	3.33	44.9-187.4	112	5156.90	1.27	3.02
B	40.6-187.1	112	5009.10	1.31	3.42	43.9-184.9	112	5082.35	1.26	3.00
C	39.9-182.6	112	4932.80	1.27	3.46	44.6-177.3	112	5011.65	1.18	3.78

\* In this table, for comparative purposes, the data for the individually-fed pigs are accorded the same treatment as those for the group-fed pigs, the periods required for the attainment of 90 and 150 lb. L.W. being determined by the dates on which the pigs averaged these two weights. See Table VIII for more precise data for individually-fed pigs.

Table VIII contains a detailed analysis of the data for the individually-fed pigs, the results being arranged so as to enable the influence of the feeding treatments to be compared not only over the period of the whole trial, but also for the periods represented by the following increments of live weight: up to 90 lb., 90-150 lb., 150-200 lb. A somewhat different treatment of the data for the individually-fed pigs is shown

Table VIII. Data for individually-fed pigs assembled according to treatment

Pen	No. of pig	Sex	Up to 90 lb. L. W.					90-150 lb. L. W.						
			lb. L. W. on 5 Feb.	Date 90 lb. attained	No. of days re-quired	lb. L. W. per day	lb. meal consumed	lb. meal per lb. L. W. I.	Date 150 lb. attained	No. of days re-quired	lb. L. W. per day	lb. meal consumed	lb. meal per lb. L. W. I.	
Treatment A														
I	993	Gilt	357	48½	16 Mar.	39	1.06	123.55	2.98	26 Apr.	41	1.46	208.05	3.46
I	988	Hog	"	38½	26 "	49	1.05	143.50	2.79	2 May	37	1.62	185.60	3.09
II	1004	Gilt	327	32½	31 "	54	1.06	150.95	2.63	8 "	38	1.58	192.90	3.22
II	1013	Hog	"	35	1 Apr.	55	1.00	158.70	2.89	16 "	45	1.33	228.70	3.81
III	997	Gilt	342	35	30 Mar.	53	1.04	152.25	2.77	7 "	38	1.58	192.85	3.21
III	999	Hog	"	41	24 "	47	1.04	133.40	2.72	4 "	41	1.46	206.75	3.45
IV	970	Hog	208	48	12 "	35	1.20	106.85	2.54	19 Apr.	38	1.58	191.60	3.19
IV	968	Gilt	"	45½	14 "	37	1.20	113.00	2.54	21 "	38	1.58	191.00	3.18
V	947	Gilt	437	32½	3 Apr.	57	1.01	157.60	2.74	15 May	42	1.43	213.90	3.57
V	949	Hog	"	43	18 Mar.	41	1.15	126.40	2.69	25 Apr.	38	1.58	192.30	3.20
			Means	39.9		46.7	1.08	136.62	2.73		39.6	1.52	200.37	3.34
Treatment B														
I	989	Gilt	357	48	12 Mar.	35	1.20	109.90	2.62	23 Apr.	42	1.43	211.40	3.52
I	991	Hog	"	39	29 "	52	0.98	155.55	3.05	11 May	43	1.40	218.60	3.64
II	1010	Gilt	327	32½	29 "	52	1.11	145.60	2.53	9 "	41	1.46	208.00	3.47
II	1008	Hog	"	38	22 "	45	1.15	131.95	2.54	30 Apr.	39	1.54	196.00	3.27
III	998	Gilt	342	41	25 "	48	1.02	138.05	2.82	3 May	39	1.54	197.45	3.29
III	996	Hog	"	45½	25 "	48	0.93	142.30	3.20	8 "	44	1.36	222.60	3.71
IV	967	Gilt	208	46	20 "	43	1.02	123.75	2.81	30 Apr.	41	1.46	205.70	3.43
IV	966	Hog	"	39½	26 "	49	1.03	147.70	2.92	8 May	43	1.40	219.60	3.66
V	946	Gilt	437	37	25 "	48	1.10	139.40	2.63	2 "	38	1.58	190.40	3.17
V	945	Hog	"	39½	25 "	48	1.05	144.65	2.86	4 "	40	1.50	201.85	3.36
			Means	40.6		46.8	1.06	137.89	2.80		41.0	1.46	207.16	3.45
Treatment C														
I	987	Gilt	357	48½	12 Mar.	35	1.18	109.90	2.65	25 Apr.	44	1.36	222.45	3.71
I	994	Hog	"	47½	16 "	39	1.09	125.30	2.95	30 "	45	1.33	228.90	3.81
II	1003	Gilt	327	28½	6 Apr.	60	1.02	157.75	2.57	18 May	42	1.43	213.70	3.56
II	1007	Hog	"	37	28 Mar.	51	1.04	150.00	2.83	11 "	44	1.36	222.75	3.71
III	995	Gilt	342	33	9 Apr.	63	0.90	174.35	3.06	23 "	44	1.36	222.00	3.70
III	1001	Hog	"	41½	24 Mar.	47	1.03	139.95	2.88	6 "	43	1.40	219.05	3.65
IV	963	Gilt	208	50½	12 "	35	1.13	108.95	2.76	22 Apr.	41	1.46	206.20	3.44
IV	964	Hog	"	42½	18 "	41	1.16	126.40	2.66	30 "	43	1.40	217.65	3.63
V	950	Gilt	437	30½	4 Apr.	58	1.03	156.80	2.63	17 May	43	1.40	217.15	3.62
V	951	Hog	"	39½	26 Mar.	49	1.03	145.95	2.89	8 "	43	1.40	217.15	3.62
			Means	39.9		47.8	1.06	139.53	2.79		43.2	1.39	218.70	3.65

Table VIII (continued)

Pen	No. of pig	Sex	Litter	150-200 lb. L. w.						Whole trial			
				Date 200 lb. attained	No. of days required	lb. per day	lb. meal consumed	lb. per day	No. of days on exp.	lb. L. w. I.	lb. meal consumed	lb. L. w. I.	
I	993	Gilt	357	25 May	29	1-72	189-70	3-79	109	151-5	521-30	3-44	
I	988	Hog		7 June	36	1-39	234-95	4-70	122	161-5	564-05	3-49	
II	1004	Gilt	327	10 "	33	1-52	216-55	4-33	125	167-5	560-40	3-35	
II	1013	Hog	"	22 "	37	1-35	232-55	4-65	137	165	619-95	3-76	
III	997	Gilt	342	10 "	34	1-47	219-80	4-40	125	165	564-90	3-42	
III	999	Hog	"	7 "	34	1-47	222-25	4-45	122	159	562-40	3-54	
IV	970	Hog	208	21 May	32	1-56	209-50	4-19	105	152	507-95	3-34	
IV	968	Gilt	"	18 "	27	1-85	176-60	3-53	102	154-5	480-60	3-11	
V	947	Gilt	437	15 June	31	1-61	203-35	4-07	130	167-5	574-85	3-43	
V	949	Hog	"	24 May	29	1-72	190-05	3-80	108	157	508-75	3-24	
	Means				32-2	1-56	209-53	4-19	118-5	160-1	546-52	3-41	
Treatment B													
I	989	Gilt	357	25 May	32	1-56	210-00	4-20	109	152	531-30	3-50	
I	991	Hog		16 June	36	1-39	235-30	4-71	131	161	609-45	3-79	
II	1010	Gilt	327	11 "	33	1-52	215-50	4-31	126	167-5	569-10	3-40	
II	1008	Hog	"	1 "	32	1-56	210-35	4-21	116	162	538-30	3-32	
III	998	Gilt	342	8 "	36	1-39	235-90	4-72	123	159	571-40	3-59	
III	996	Hog	"	13 "	36	1-39	235-45	4-71	128	154-5	600-35	3-89	
IV	967	Gilt	208	2 "	33	1-52	217-45	4-35	117	154	546-90	3-55	
IV	966	Hog	"	11 "	34	1-47	223-15	4-46	126	160-5	590-45	3-68	
V	946	Gilt	437	4 "	33	1-52	215-15	4-30	119	163	544-95	3-34	
V	945	Hog	"	6 "	33	1-52	214-90	4-30	121	160-5	561-40	3-50	
	Means				33-8	1-48	221-32	4-43	121-6	159-4	566-37	3-55	
Treatment C													
I	987	Gilt	357	26 May	31	1-61	203-00	4-06	110	151-5	535-35	3-53	
I	994	Hog		7 June	38	1-32	232-20	5-04	122	152-5	606-40	3-98	
II	1003	Gilt	327	22 "	35	1-43	229-75	4-60	137	171-5	601-20	3-51	
II	1007	Hog	"	16 "	36	1-39	235-65	4-71	131	163	608-40	3-73	
III	995	Gilt	342	29 "	37	1-35	241-55	4-83	144	167	637-90	3-82	
III	1001	Hog	"	11 "	36	1-39	237-25	4-75	126	158-5	596-25	3-76	
III	963	Gilt	208	21 May	29	1-72	183-85	3-68	105	149-5	499-00	3-34	
IV	964	Hog	"	1 June	32	1-56	210-50	4-21	116	157-5	554-55	3-32	
IV	950	Gilt	437	17 "	31	1-61	202-60	4-05	132	169-5	576-55	3-40	
V	951	Hog	"	10 "	33	1-52	214-90	4-30	125	160-5	578-00	3-50	
	Means				33-8	1-49	221-12	4-42	124-8	160-1	579-36	3-62	

in Table VII with the object of throwing light on the advantages, from the standpoints of L.W.I. and efficiency of food conversion, of feeding pigs as individuals in separate compartments instead of as groups from a common trough. For this purpose the data for the individually-fed pigs are accorded the same treatment as those for the group-fed pigs, the number of days required for the attainment of 90 and 150 lb. L.W. being reckoned by the dates on which the *average* weights of the pigs under the different treatments had reached these values. By this means the data for meal consumption, rate of L.W.I. and efficiency of food conversion for the individually-fed and group-fed pigs are placed on a more comparable basis, although, of course, there still remains the distinction that changes in ration under the individual-feeding system were effected when each pig arrived exactly at 90 and 150 lb. L.W., whereas under the group-feeding system some pigs were considerably above and some below 90 and 150 lb. L.W. when the group averages, which corresponded with the changes of ration, had reached these levels.

The methods to be adopted in the statistical analysis of the data for both the individually-fed and group-fed pigs are explained in the next section of this paper. It will be of interest at this point, however, to direct attention to certain of the conclusions to which the data in Tables V, VI, VII and VIII appear to point, referring when necessary to the findings in respect of significance that have been worked out in the appropriate section.

### *Individual-feeding results*

The average results in Table VIII for the individually-fed pigs suggest very strongly that increasing the protein supply in the ration to a very high level has had very little effect on the progress of the pigs, whether measured in days required to reach a given live weight, rate of L.W.I. per day or efficiency of food conversion. This finding is brought out more clearly by summarizing the essential data in Table IX.

Table IX. *Average results for individually-fed pigs  
(ten pigs in each treatment)*

Treatment	Up to 90 lb. L. W.				90-150 lb. L. W.			150-200 lb. L. W.			Whole trial		
	lb. L. W. on 5 Feb.	No. of days re-quired	lb. L. W. I. per day	lb. meal per lb. L. W. I.	No. of days re-quired	lb. L. W. I. per day	lb. meal per lb. L. W. I.	No. of days re-quired	lb. L. W. I. per day	lb. meal per lb. L. W. I.	No. of days exp.	lb. L. W. I. per day	lb. meal per lb. L. W. I.
A	39.9	46.7	1.08	2.73	39.6	1.52	3.34	32.2	1.56	4.19	118.5	1.35	3.41
B	40.6	46.8	1.06	2.80	41.0	1.46	3.45	33.8	1.48	4.43	121.6	1.31	3.55
C	39.9	47.8	1.06	2.79	43.2	1.39	3.65	33.8	1.49	4.42	124.8	1.29	3.62

A, normal protein; B, higher protein; C, highest protein.

The differences between the averages for the pigs under the three feeding treatments are in all cases quite small compared with the range of variation in the data for the individuals on any one treatment. This is particularly true of the first period of feeding up to 90 lb. L.W. In this period, when the influence of extra protein in improving the rate of growth might have been expected to be most clearly manifested, it is impossible, on the basis of the average results, to discern evidence of any such effect.

It may be concluded, therefore, that, up to 90 lb. L.W., ration A, containing 12 per cent of fish meal, furnished all the digestible protein required for the maximum rate of growth as conditioned by the net energy content of the ration and the inherited capacity for growth of the individual pigs. Protein supply had clearly ceased to be a limiting factor at this level, and consequently the large increases to 22 and 32 per cent of protein-rich food in rations B and C brought about no improvement, and indeed no marked change of any kind, in the rate of L.W.I. or efficiency of food conversion. Whether the L.W.I. in the pigs on treatments B and C was of a leaner nature than that in the animals on treatment A will be decided later when the post-slaughter results are dealt with.

Although the differences between the averages for the individually-fed pigs under the three feeding treatments are small compared with the individual variation within any one treatment, they are nevertheless consistent. For example, the C pigs, subsisting on the highest protein supply, required on an average 6-7 days longer to reach 200 lb. L.W. than the A pigs (normal protein supply), the B pigs (intermediate protein supply) occupying in this respect an intermediate position. In respect of daily L.W.I. and efficiency of food conversion, the average results in Table IX for the whole trial display a small, though consistent deterioration in passing from treatment A to treatment C. These small differences in favour of treatment A are traceable at all stages of the feeding trial, but most notably in the period from 90-150 lb. L.W.

Further confirmation of these findings is provided by Table VI, showing the average weekly live-weights of the pigs under the three feeding treatments over the period of comparison from 5 February to 28 May. On the latter date the individually-fed A pigs averaged 190.9 lb. L.W., the B pigs 187.1 lb. and the C pigs 182.6 lb. These differentiations in mean live-weight, occasioned, as will be shown, by the different character of the feeding and not by differences in actual weight of meal consumed, are not large, but they again show the values of the treatments from the standpoint of L.W.I. to be in the order of A, B and C.

In regard to the statistical significance of the foregoing conclusions (see statistical section) it is merely necessary at this stage to state that the small drop in L.W.I. noted in passing from the normal diet (A) to one abnormally rich in protein (C) is definitely significant, and is still significant when corrected for the different amounts of meal consumed under the two feeding treatments. It is satisfactory to record that the individual-feeding lay-out adopted in the present trial permitted of the carrying out of the comparison of the three feeding treatments with an accuracy comparable with that associated with modern replicated experiments on agricultural crops.

The general conclusion may be drawn, therefore, that a feeding treatment providing 12 per cent of fish meal from weaning to 90 lb. L.W., 10 per cent of fish meal from 90 to 150 lb. and 5 per cent of a mixture of equal parts of meat meal and ex. soya-bean meal in the final period will supply all the digestible protein required for the maximum rate of growth permitted by the net energy content of the ration. An increase in the protein supply beyond these levels does not lead to an increased rate of L.W.I. Indeed, if the level of protein supply be raised substantially, a small though significant depression in the rate of L.W.I. and a slight increase in the amount of meal required per lb. L.W.I. are occasioned, this being particularly noticeable in the period from 90–150 lb. L.W. Unless, therefore, the high levels of protein intake lead to an improvement in bacon quality by giving rise to a leaner carcass, there is nothing to be gained, and much to be lost from the financial standpoint, by feeding higher amounts of protein-rich food than the normal levels supplied in treatment A. The question of carcass quality in relation to the feeding treatments will be dealt with in a later section.

Although the foregoing conclusion may possibly be applicable to pig-feeding in the widest sense, it is only fair to point out that, strictly speaking, it is referable only to the particular strain of Large Whites forming the experimental animals in the present trial, and possibly only to animals of this strain in so far as they subsist on rations made up from the foods used in the present feeding treatments. It is not justifiable at this stage to assume that all the bacon breeds display the same quantitative behaviour in respect of utilization of protein.

Since the small differences in the mean rates of L.W.I. are a significant effect of the feeding treatments (i.e. are not due merely to differences in the average amounts of meal consumed by the pigs on the three treatments) it is reasonable to anticipate that they may be due, at least in part, to a slight falling-off in the net energy content of the rations in the direction of A to C. In all probability this is the case, since the

B rations are derived from the A rations by substituting a given percentage of barley meal (starch equivalent as ordinarily computed = 71.4) by an equal percentage of the mixture of equal parts of meat meal and ex. soya-bean meal (starch equivalent of mixture = 59), whilst the C rations are derived by a similar replacement of twice the amount of barley meal (see Table I).

### *Group-feeding results*

It now remains to inquire whether the findings of the individual-feeding trial receive any confirmation from the results obtained by the group-feeding method. An inspection of Tables VI and VII shows that this was the case. Again the differences between the average weights of the pigs under the three treatments were small at the conclusion of the comparison on 28 May (Table VI) compared with the individual variation among the pigs under any one treatment (Table V). The mean live-weights on 28 May of the group-fed pigs under treatments A, B and C were 187.4, 184.9 and 177.3 lb. respectively, the actual mean gains in live-weight over the period of comparison being 142.5, 141.0 and 132.7 lb. respectively. These results show a general trend very similar to that of the corresponding averages for the individually-fed pigs, namely, 190.9, 187.1 and 182.6 lb. for the mean live-weights on 28 May and 151.0, 146.5 and 142.7 lb. for the mean gains from 5 February to 28 May. Moreover, the comparative data in Table VII for the mean daily rate of L.W.I. and for the efficiency of food conversion, both over the entire trial and at the different stages of the trial, again demonstrate the general support afforded by the group-feeding results to the conclusions already drawn from the individual-feeding test.

Reference to the statistical section that follows, however, reveals the fact that owing to the relative insensitivity of the group-feeding lay-out, the differences in mean L.W.I. (Table VI) resulting from the different feeding treatments are not, in the case of the group-fed pigs, statistically significant. Owing to the smallness of the differences, it is not possible to prove that they are definitely assignable to differences in feeding treatment. Clearly the group-feeding technique lacks the sensitiveness of the individual-feeding method. Nevertheless, it is of particular interest that the small differences related to feeding treatment, and demonstrably significant by the individual-feeding technique, are brought to light also, though not significantly so in the statistical sense, by the group-fed animals. This in itself is sufficient justification for the dual method

adopted in the present investigation. If differences exist between feeding treatments, they should be detectable and their magnitude be measurable by a suitably designed individual-feeding lay-out; but unless such differences are capable of being observed in group-feeding trials, they must lack actual practical significance, since the pig-feeder rations his animals in groups and not as individuals. The group-feeding trial is thus the link between practical feeding and scientific experimentation and may indeed be regarded as a verification under practical conditions of the significance of the results of the individual-feeding trial. By itself, when the expected differences to be investigated are small, the group-feeding technique may be of very limited value. When carried out side-by-side with the individual-feeding trial, however, it may, as in the present experiment, constitute an important confirmation.

*Thriftiness of pigs under conditions of group feeding  
and individual feeding*

It will be interesting at this stage to compare the thriftiness of the pigs on the same feeding treatments under conditions of group feeding and individual feeding. The relevant data are in Tables VI and VII. From 5 to 19 February there was a tendency for the individually-fed pigs to display less thriftiness than the group-fed animals. This may have been due to the slightly higher initial average live weights of the group-fed pigs tending to lead to slightly higher average gains, but a contributory factor may have been the disturbance caused by training the individually-fed pigs to enter their proper compartments at feeding time. During the subsequent period, however, the individually-fed pigs gradually overhauled the group-fed pigs, and at the conclusion of the comparison on 28 May it was found that on the treatments A, B and C they had averaged 8.5, 5.5 and 10 lb. per head greater gains than the group-fed pigs. This greater thriftiness of the pigs under individual treatment is also shown by the number of days required for the animals to arrive at slaughter weight. The individually-fed pigs on the treatments A, B and C averaged 107.8, 112.0 and 114.3 days respectively to grow from 49 to 200 lb. L.W., the corresponding average periods for the group-fed pigs being 116.6, 116.5 and 122.8 days.

The poorer thriftiness of the pigs fed as groups, the statistical significance of which is demonstrated later, is to be ascribed to the energy expended by the animals in the struggling that takes place at feeding time when a group of pigs is fed from a common trough. The smaller, weaker animals under such feeding conditions are frequently unable to



secure their fair share of the meal and their progress is thereby unduly slowed down, a circumstance that further prejudices the index of thriftiness of the group as a whole. On the other hand, each individually-fed pig is supplied with a correct ration based on live-weight and is enabled to consume this ration without unnecessary expenditure of energy.

The comparative data in Table VII show that on the A treatment, the group-fed animals consumed per ten pigs, over the whole period, 127.75 lb. more meal than the individually-fed pigs, despite the fact that they averaged 8.5 lb. less L.W.I. per head. On treatment B, they consumed 73.25 lb. more meal per ten pigs, but averaged 5.5 lb. L.W.I. less per head, while on treatment C, the extra meal consumption per ten group-fed pigs was 78.85 lb. with an average of 10 lb. L.W.I. less per head.

Among the individually-fed pigs, those on treatment A averaged the highest L.W.I., namely, 151 lb., between 5 February and 28 May. Over this period, the ten A pigs consumed 5029 lb. of meal. From the data in Tables V and VI it is possible, by assuming simple proportionality, to calculate approximately what the meal consumption per ten pigs would have been in each of the other lots for the same average L.W.I. Thus, the ten group-fed pigs on treatment A consumed a total of 5157 lb. of meal for an average L.W.I. of 142.5 lb., so that for a L.W.I. of 151 lb. the meal consumption would have been approximately 5465 lb. The computed comparative data are shown in Table X.

Table X. *Meal consumption per ten pigs for an average L.W.I. of 151 lb.*

	Treatment A lb. meal	Treatment B lb. meal	Treatment C lb. meal
Group-fed pigs	5465	5443	5703
Individually-fed pigs	5029	5163	5220
Difference per 10 pigs	436	280	483
Mean difference per 10 pigs	399.7		

Taking into account the data for the whole sixty pigs, therefore, it will be seen from Table X that in rearing the animals from an average live weight of 42.3 lb. to an average of 193.3 lb., the individual method of feeding led to an average saving of 40 lb. of meal per head, i.e., 7.2 per cent of the average meal consumption per head of the group-fed animals. Some part of this saving is no doubt to be attributed to the closer control of feeding (in respect both of amount of food and in being

able to make the necessary changes of ration on the precise dates at which the animals arrived at 90 and 150 lb. L.W.) that is possible when pigs are fed separately, but it is probable that in the main, the extent of the saving may be taken as a measure of the energy expended by the group-fed pigs in consequence of the "scrambling" at feeding time resulting from feeding from a common trough. The figure is naturally not of general application, but has reference strictly to the present conditions of management and to the characteristics of the strain of Large Whites used in the experiment. Since the group-fed pigs in this trial were given ample trough space, it follows that under conditions of over-crowding and insufficient trough space, the energy expended in struggling for food at meal times might lead to an increase in the meal requirement for a given L.W.I. to a figure considerably higher than that noted in the present trial. It is realized that individual feeding of a commercial herd, with the object of reducing to a minimum such wasteful expenditure of muscular energy at feeding time, is not feasible, but the results are important in that they emphasize the need of avoiding over-crowding. Struggling to secure food from a trough of insufficient dimensions may obviously lead to a serious decrease in the efficiency of food conversion, and failure to pay attention to this factor may render pig-feeding uneconomic.

Further features of interest, such as the influence of sex on the rate of L.W.I. and efficiency of food conversion and the effect of initial weight on L.W.I., are dealt with in the statistical section that follows.

#### STATISTICAL STUDY OF THE PRE-SLAUGHTER DATA<sup>1</sup>

The function of the statistician in relation to experiments, of whatever kind, is (a) to see that the experiment is so designed that valid deductions are possible on the questions at issue, and (b) to analyse the resulting data in order to see what significant conclusions, if any, have emerged. The design will depend upon the magnitude of the differences to be expected as a result of treatment; a crude experiment will be enough if quite large differences are anticipated, but if the expected differences are small more refined methods will be necessary. This point is well brought out by a preliminary study of the data of the group-feeding trial. It will be convenient to consider pre-slaughter and post-slaughter results separately. The latter will be reported on later in the paper as they come up for discussion. In this section we shall be concerned with

<sup>1</sup> By Dr J. Wishart.

the effect of the different feeding treatments on the growth of the pigs. Pre-slaughter records are available on the following points:

- Weight at weaning.
- Initial weight when trial commenced.
- Weekly weights during the trial.
- Food consumption.

For the present purpose it was considered sufficient to calculate the live-weight increase over the 16 weeks of the trial, i.e. the difference between initial and final weights. Sex and litter are known for each pig.

In the group trial there were three large pens for the three feeding treatments, A, B and C. Each pen contained ten pigs, six hogs and four gilts, not all from the same litter. These were fed a common ration, but weighed individually. The food consumption being known only for the whole pen, only a very crude comparison is possible between the treatments on the question of efficiency of meal conversion. On the other points listed above individual records are available. The important thing is to investigate what differences, if any, in live-weight gain have emerged from feeding groups of pigs with meal having different protein content. For this purpose we have thirty records of live-weight gain over 16 weeks, in three groups of ten each. On the assumption that, apart from a possible effect of treatment, pigs in different pens are not affected by other than random variations which equally affect pigs within the same pen, we can ascertain the effect of treatment by means of the following simple analysis of variance:

Table XI. *Analysis of variance of live-weight gain ( $\frac{1}{2}$ -lb. units)*

Variation	Degrees of freedom	Sum of squares	Mean square
Between treatments	2	2220.1	1110.0
Within treatments	27	32369.3	1198.9
Total	29	34589.4	

It is obvious from this table, without further specific test, that the differences between treatments in live-weight gain are not significant, the variation being of the same order as the random variation to be expected with this form of lay-out between undifferentiated groups of ten pigs.

The standard error for each pig's live-weight gain is  $\sqrt{(1198.9)}$ , or 34.6  $\frac{1}{2}$  lb., which is 12.5 per cent of the mean live-weight gain, 277.4  $\frac{1}{2}$  lb. For the groups (means of ten pigs) we have the following summary results:

Table XII. *Summary of results. Group-fed pigs*

Mean live-weight gain	A	B	C	Mean	Standard error
$\frac{1}{2}$ lb. per 16 weeks	285.0	281.9	265.4	277.4	10.95
lb. per day	1.27	1.26	1.18	1.24	0.05
Percentage	102.7	101.6	95.7	100.0	3.95

Although these figures suggest that the live-weight gain is less where the high protein diet has been used, the experiment is evidently too crude to detect this, the drop from A to C being less than twice the standard error. The principal effect<sup>1</sup> is the drop from 285.0 to 265.4, and this can be isolated in a single degree of freedom out of the two between treatments, the sum of squares being

$$(2850 - 2654)^2/20 = 1920.8.$$

The mean square is also 1920.8, still quite insignificant when compared with the mean square within treatments.

That sex differences in live-weight gain do not exist is brought out by analysing the data further, as between hogs and gilts. Although the numbers are unequal, the 6 to 4 proportion is fixed for all treatments, and this enables us to divide the variation between the six groups into a treatment effect, a sex difference and the interaction of these factors, as follows:

Table XIII. *Effect of sex in relation to treatment*

Variation	Degrees of freedom	Sum of squares	Mean square
Treatments	2	2220.1	1110.0
Sex	1	929.3	929.3
Interaction	2	5868.1	2934.0
Total between groups	5	9017.5	1803.5
Within groups	24	25571.9	1065.5

There are no significant differences among the mean squares of this table. A standard error might be calculated from the last line of the table, but its value would be only slightly smaller than the figure we used above, the reduction being due to the fact that the "interaction" of the table is somewhat abnormal, though not significantly so.

Now it has been stated more than once in statistical publications<sup>2</sup> that an improvement in precision with animal experimentation may be

<sup>1</sup> R. A. Fisher, *The Design of Experiments*, 1935, p. 132.

<sup>2</sup> See, e.g. R. A. Fisher, *Statistical Methods for Research Workers*, 1934 (5th. ed.), § 49, 1, or J. Wishart & H. G. Sanders, *Principles and Practice of Field Experimentation*, 1935 (Emp. Cott. Grow. Corp.), p. 45.

expected if initial ages and weights are taken account of, and a correction made so as to give effectively the live-weight gains that would have been shown had all the animals commenced their diets at the same age and weight. The method is to work out the regression of live-weight gain on initial age and weight and use the technique of the analysis of covariance. In the present case the ages were practically identical for all pigs, and need not be further considered, but there were considerable variations in initial weight on 5 February, the date when the pigs may be said to have settled down to their differential feeding treatments. If we take initial weight into account as a correlated variable with the present group-fed data we find, however, that the regression of live-weight gain on initial weight is not significant, as shown in the following table:

Table XIV. *Regression of live-weight gain on initial weight*

Variation	Degrees of freedom	Sum of squares	Mean square	$z=0.6601$
Regression	1	4074.1	4074.1	
Deviations	26	28295.2	1088.3	
Total	27	32369.3		

In this table the sum of squares for error from Table XI is split up into the part due to the regression and the remainder. The value of  $z$ , obtained by calculating one-half the natural logarithm of the ratio of the mean squares, is 0.6601, but a value of 0.7205 would be needed to give significance at the 5 per cent level of probability (see Fisher's  $z$ -table). Actually the value of the regression coefficient is 0.719, corresponding to a correlation coefficient of +0.355. In view of this lack of significance we can hardly expect an increase in precision through bringing in the correlated variable, and in fact the error mean square is only reduced very slightly from 1198.9 to 1088.3. The calculations will not, therefore, be proceeded with. This low correlation with initial weight may be due to competition at the feeding trough, for, as we shall see, much better results are obtained from the individual-feeding data. It is worth noting that the correlation with weaning weight is even less than the above figure ( $r = +0.294$ ).

There is little more that can be done with the group-feeding data, and we now turn our attention to the individual trial, where more precise results are to be expected. In this trial there were five pens, each given over to six pigs (three hogs and three gilts), all drawn from the same litter. One lot of three pigs, with one exception either all hogs or all gilts, was composed of animals definitely under average weight at

weaning, while the other lot was over average weight. The pigs within each lot were allotted at random to the three feeding treatments, so that one hog and one gilt from each pen had each of the three treatments. The pigs were fed by means of individual-feeding boxes, separate records of weight and meal consumption being kept. This experimental arrangement permits of a close comparison between pigs of the same litter and nearly the same weaning weight in respect to treatment, but it has one possible drawback, in that the sex comparison has been confounded with the comparison on the basis of weaning weight in a way which makes it impossible to say with certainty that differences between the hog and gilt groups are really due to sex. As sex differences due to treatment are considered of interest, especially with the post-slaughter results, this is a point to take note of in future experimental work. Meantime we may observe that a correction for weaning or initial weight by covariance will enable sex differences to be examined by means of an analysis of the residual variation after adjustment. It may be doubted whether, in view of this possibility, it was necessary for the pigs to have been selected at all in the first instance on the basis of weight. Calculations bearing on this point will be given in due course. A simple suggested scheme would be to allot two pigs (one of each sex) to each of the three treatments, the whole arrangement being a random one.

Individual weaning weights are on record, as also the initial weights on 5 February. Thereafter, weekly weighings were made until the pig reached 200 lb., when it was ready for slaughter. The minimum period under experiment was 16 weeks to 28 May, and live-weight gains were calculated over this period. The total meal consumption of each pig for this period is also known.

#### *Analysis of results*

The greatest degree of heterogeneity to be looked for in the data of live-weight gain may be expected to be due to the different litters used. The variation on this account is part of that between the groups of three pigs, and may be assessed by separating the pen variation, with 4 degrees of freedom, from the variation between groups, which has 9 degrees of freedom. The single degree of freedom for the heavy-light comparison may also be isolated at this point. Whatever differences there are on the foregoing counts should make no difference to the accuracy of the experiment, for the feeding comparisons are made within groups of three, and this variation, having 20 degrees of freedom, can be worked out, and the part due to treatments, with 2 degrees of freedom, separated from it.

A further part, conceivably of interest and worth looking at, is the interaction of treatments with the heavy and light groups. Whatever is left, in both parts of the analysis, may be taken to be a measure of random variation. The complete analysis of variance is as follows:

Table XV. *Analysis of variance of live-weight gain in  $\frac{1}{2}$  lb.*

Variation	Degrees of freedom	Sum of squares	Mean square
Pens	4	4189.87	1047.47
Heavy-light	1	580.80	580.80
Interaction	4	948.53	237.13
Between groups	9	5719.20	635.47
Treatments	2	1398.20	699.10
Interaction with heavy-light	2	312.20	156.10
Error	16	6769.60	423.10
Within groups	20	8480.00	424.00
Total	29	14199.20	

The first thing to note is that the mean squares between and within groups are not significantly different. This was also found to be the case with weaning and initial weights, and with the meal consumed. It was considered justifiable, therefore, to proceed with a simplified form of analysis, in which a single measure of error was used, based on 22 degrees of freedom, since the interaction of treatments with the heavy and light groups was thrown in, having been found to be insignificant in all cases. If at the same time we isolate the principal effect of treatment, already referred to in the case of the group-fed data, we get the following table:

Table XVI. *Analysis of variance of live-weight gain in  $\frac{1}{2}$  lb.*

Variation	Degrees of freedom	Sum of squares	Mean square	<i>z</i>
Pens	4	4189.87	1047.47	0.5272 S
Treatments: Main effect	1	1394.45	1394.45	0.6701 NS
Rest	1	3.75	3.75	
Heavy-light	1	580.80	580.80	
Error	22	8030.33	365.02	
Total	29	14199.20		

S=significant at 5 % point; NS=not significant.

The first thing to note about this table is the great increase in precision over the group-fed data. The standard error for each pig's live-weight gain is  $\sqrt{(365.02)}$ , or 19.1  $\frac{1}{2}$  lb., which is 6.5 per cent of the mean live-weight gain, 293.4  $\frac{1}{2}$  lb. The corresponding figure for the group trial was 12.5 per cent. Precision being measured in terms of variance, or the square of the standard error, we see that the individual trial is nearly

four times as accurate as the corresponding group trial. It is interesting to note that the figure of 6.5 per cent compares very favourably with the usual figures for standard error per plot in the case of modern replicated experiments on agricultural crops. Our experiment has shown, therefore, that with careful designing animal experiments can be every bit as accurate as field trials.

In spite, however, of the very satisfactory level of precision reached, very little has emerged in the way of significant conclusions respecting the differences between the three treatments. The pen differences, probably due to different growth rates in the case of different litters, are just significant at the 5 per cent level of probability, but even although practically the whole of the treatment variation has been isolated in the 1 degree of freedom representing the drop from A to C, this effect is not significant. For  $n_1=1$ ,  $n_2=22$ ,  $z$  would have to be 0.7294 for significance at the 5 per cent level. Provisionally, therefore, we have to conclude that the smaller live-weight gain with high protein feeding is not demonstrated beyond doubt. The difference between the heavy and light groups is evidently not significant, and the main results of the experiment may be presented in the following summary table:

Table XVII. *Summary of results. Individually-fed pigs*

Mean live-weight gain	A	B	C	Mean	Standard error
$\frac{1}{2}$ lb. per 16 weeks	302.0	292.9	285.3	293.4	6.04
lb. per day	1.35	1.31	1.27	1.31	0.03
Percentage	102.9	99.8	97.2	100.0	2.06

The drop in live-weight gain from A to C is 5.7 per cent, an amount which, as we have seen, is scarcely large enough to be significant, judging from the variation between pigs of the same litter treated alike. Its standard error is  $2.06 \sqrt{2}$ , or 2.9. It is worth noting that the mean live-weight gain of the individually-fed pigs is significantly greater than that of the group-fed pigs. Accepting the standard errors given in Tables XII and XVII as appropriate for the two classes, and dividing by  $\sqrt{3}$  to obtain the standard errors for the means of thirty pigs, we find that the difference between 1.31 and 1.24 lb. per day is nearly four times its standard error. The initial weights of the group-fed pigs were some 10 per cent higher than those of the individually-fed pigs, but this cannot be taken as an explanation, for the correlation between live-weight gain and initial weight is positive. This correlation was not significant in the case of the group-fed data, but it is numerically larger, and significant, in the case of the individual data. Thus we should expect



a higher live-weight gain with the group-fed data in consequence of higher initial weights, but the reverse is the case. The total amount of meal consumed was about the same in both cases, the average per pig being 499 lb. for the individually-fed pigs and 508 lb. for those fed in groups. It appears, therefore, that feeding the pigs individually has proved effective in getting the animals ready for the bacon factory at a distinctly earlier date than those fed in groups, without any loss in efficiency of meal conversion over the period of the trial.

An examination of the figures for weaning and initial weights in the individual trial was now undertaken. The first point noted was that the regression of live-weight gain on weaning weight was significant, the coefficient being 2.186, corresponding to a correlation coefficient of +0.486. On correction of live-weight gain for variations in weaning weight, however, it was found that the drop from A to C could still not be regarded as significant. For this reason the details of the calculation will not be given. It will suffice to say that the error mean square for live-weight gain was reduced from 365.0 to 292.1, and that the principal effect on the adjusted data showed a drop from A to C of 5.2 per cent in live-weight gain, which drop had a standard error of 2.6 per cent. The corresponding figures for the unadjusted data were 5.7 and 2.9.

In the case of initial weight the result is more satisfactory. The full table of analysis of variance and covariance follows, in which initial weight is denoted by  $x$ , and live-weight gain by  $y$ , the unit in both cases being a  $\frac{1}{2}$  lb. one.

Table XVIII. *Analysis of variance and covariance.*  
*Live-weight gain and initial weight*

Variation	Degrees of freedom	( $x^2$ )	( $xy$ )	( $y^2$ )	( $xy$ )/( $x^2$ )
Pens	4	2390.13	2141.57	4189.87	
Treatments: Main effect	1	0.05	8.35	1394.45	
Rest	1	12.15	-6.75	3.75	
Heavy-light	1	1190.70	831.60	580.80	
Error	22	689.27	1407.63	8030.33	2874.70
Total	29	4282.30	4382.40	14199.20	

The error regression coefficient ( $b$ ) of live-weight gain on initial weight is 2.042, and corresponds to a correlation coefficient of +0.598. The last column contains the part of the error sum of squares due to the regression, with 1 degree of freedom, the calculation being as shown. That the regression is significant is seen by comparing this part with the remainder, as follows:

Table XIX. *Test of significance of regression coefficient*

Variation	Degrees of freedom	Sum of squares	Mean square	<i>z</i>
Regression	1	2874.70	2874.70	1.2302 SS
Deviations	21	5155.63	245.51	
Total error	22	8030.33		

SS = significant at 1 % point.

The value of *z* is significant at the 1 per cent level, and the error mean square has been reduced from 365.0 to 245.5. The latter figure yields a standard error for each pig's live-weight gain of  $15.7 \frac{1}{2}$  lb., or 5.3 per cent, as compared with 6.5 per cent before correction.

The significance of the main treatment effect, when correction is made for regression on initial weight, is now established as follows. Calculate the regression coefficient from the line "main effect" (1 degree of freedom), i.e.  $8.35/0.05$  or 167, and subtract from this the error coefficient, 2.0422. Square the difference, multiply by the product of 0.05 and 689.27, the respective sums of squares for initial weight, and divide by their sum, 689.32. The result is 1360.46, having 1 degree of freedom. This is then compared with the error residual, as in the following table:

Table XX. *Analysis of residual variance*

Variation	Degrees of freedom	Sum of squares	Mean square	<i>z</i>
Main effect	1	1360.46	1360.46	0.8561 S
Error	21	5155.63	245.51	
Total	22	6516.09		

Alternatively the total 6516.09 could be determined from the sums of squares and products for the 23 degrees of freedom, main effect + error by making the calculation  $(y^2) - (xy)^2/(x^2)$  from these sums. The residual main effect is then obtained by subtracting the residual error sum of squares. This is the general method to follow when there is more than 1 degree of freedom in the effect examined. The *z* of the above table is significant at the 5 per cent level. For  $n_1 = 1$ ,  $n_2 = 21$ , *z* should be 0.7322 at 5 per cent and 1.0408 at 1 per cent. Now the mean initial weights for the A and C groups are nearly equal at 79.9 and  $79.8 \frac{1}{2}$  lb. respectively, so the significance is evidently due to the reduction of the error by the process of adjusting individual live-weight gains for the varying initial weights. In fact the percentage drop from A to C is now 5.6 per cent, with a standard error of 2.4, as compared with the unadjusted drop of

5.7 per cent, which had a standard error of 2.9. The new percentage drop is obtained as follows:

Treat- ment	Mean initial weight ( $x$ )	$x - \bar{x}$	$b(x - \bar{x})$	Mean live- weight gain ( $y$ )	$y - b(x - \bar{x})$
A	79.9	-0.4	-0.82	302.0	302.8
B	81.2	0.9	1.84	292.9	291.1
C	79.8	-0.5	-1.02	285.3	286.3
Mean	$\bar{x} = 80.3$	—	—	$\bar{y} = 293.4$	293.4

The figures in the last column, when expressed as percentages of the general mean, 293.4, give 103.2, 99.2 and 97.6 respectively, and the difference of the first and last is 5.6. The variance of the actual drop of 16.5 from A to C in the adjusted figures is estimated by multiplying 245.51, the error residual, by 2/10, since it is a difference of two means of ten observed values. The correction to this estimate owing to the fact that the regression coefficient  $b$  is not accurately known, but is only estimated from the data, is in this case entirely negligible, owing to the agreement of the mean initial weights.<sup>1</sup> We thus get 49.1, giving for the standard error of the difference, 7.01, which is 2.4 per cent of the mean live-weight gain, 293.4  $\frac{1}{2}$  lb.

The above adjusted mean live-weight gains are the best figures we are likely to obtain from the available data, and point to the general conclusion that there is a small but significant drop in live-weight gain as we pass from a normal diet to one abnormally rich in protein. Any justification for the rich-protein diet must therefore be sought in the quality of the carcass, which must be such as to make up, at least, for the extra time and food required to produce the 200-lb. pig, the extra time being practically 1 week on the average, as the data show. Post-slaughter results are dealt with elsewhere in this paper.

The effectiveness of a correction for varying initial weight is well brought out by repeating the above calculations ignoring the heavy-light comparison. If in all that we have done the sums of squares and products for the 1 degree of freedom "heavy-light" are included in the error, to give 23 degrees of freedom for the latter, and the covariance analysis is carried out as before, it is found that the main effect is again significant, with a  $z$  of 0.8134, only slightly smaller than the former value. The error mean square is reduced to 270.2, giving a standard error of 5.6 per cent, which compares favourably with the former figure of 5.3. The regression coefficient is 1.191, corresponding to a correlation coefficient of +0.557, while the previous figure was +0.598. Thus, although the heavy-light

<sup>1</sup> For the exact formula see Wishart and Sanders, *loc. cit.*

comparison is, as is natural, very significant on initial weight, to ignore it in the covariance calculation makes very little difference in the final result. It appears, therefore, that the grouping into two classes by weight at the beginning of the experiment has not contributed materially to the efficiency of the lay-out, while correction of final figures by regression on initial weight has been necessary in order to demonstrate the significance of the differences in live-weight gain which have resulted from the differential feeding treatments.

A point remaining to be dealt with concerns the efficiency of food conversion for the different rations. There are records of the meal consumed per pig for each week during which the experiment was in progress. An analysis of variance of the 16-week totals, on the foregoing lines, gave the following results:

Table XXI. *Analysis of variance. Meal consumption in lb.*

Variation	Degrees of freedom	Sum of squares	Mean square	<i>z</i>
Pens	4	18617.3	4654.3	1.0253 SS
Treatments	2	524.6	262.3	
Heavy-light	1	7114.8	7114.8	1.2376 SS
Error	22	13175.3	598.9	
Total	29	39432.0		

As is to be expected, there is a close correlation between meal consumed and initial weight. The pens (i.e. litters) differ significantly in meal consumption, as do also the heavy and light groups. On the other hand the differences in actual amount of meal consumed in the diets A, B and C over the standard 16-week period are far from significant, even when the principal effect is considered. The averages are as follows:

Table XXII. *Summary of results. Meal consumption*

Meal consumed	A	B	C	Mean	Standard error
lb. per pig	502.9	500.9	493.2	499.0	7.74
Percentage	100.8	100.4	98.8	100.0	1.55

The drop from A to C is only 2 per cent, which is quite insignificant, even with a very low standard error. Thus over the period considered the pigs on the three diets have eaten practically equal total amounts of meal, yet those having the C treatment have put on significantly less weight than those with a normal diet, the B treatment occupying an intermediate position. A consequence of the pigs on a rich protein diet taking longer to reach 200 lb. is that they eat more meal, at a propor-

tionately greater cost, than pigs on a normal diet. If we divide the figures of Table XXII by those of Table XVII, paying attention to units, we obtain for the amount of meal consumed (in lb.) per lb. of live-weight gain the following results:

	A	B	C	Mean
lb. meal per lb. live-weight gain	3.33	3.42	3.46	3.40

Such figures are good average indices of efficiency of food conversion. On further enquiry, however, it is evident that the same relative state of affairs does not exist between the treatments at all stages of growth. Similar figures for meal consumed per lb. of live-weight gain were worked out for the stages (a) up to 90 lb. live weight, (b) 90–150 lb. live weight and (c) 150–200 lb. live weight. To complete (c) for all pigs the data have been extended where necessary beyond the 16 weeks' calendar period to which we have hitherto restricted ourselves. The ratios were calculated for individual pigs, and so it was possible to carry through an analysis of variance, with the following summary results:

Table XXIII. *Lb. meal per lb. live-weight gain*

	A	B	C	Mean	Standard error
Up to 90 lb.	2.73	2.80	2.79	2.77	0.05
90–150 lb.	3.34	3.45	3.65	3.48	0.06
150–200 lb.	4.19	4.43	4.42	4.35	0.09
Whole trial	3.41	3.55	3.62	3.53	0.05
(up to 200 lb.)					

Over the whole period of the trial (last line of table) moderately significant differences were found between the treatments. The figures, in fact, correspond to those given above for the 16-week period. Litter differences were insignificant, and the heavy and light lots were practically identical on the average of all treatments. For the period up to 90 lb. the treatment differences are small, and quite insignificant; this applies also to litter and heavy-light differences. From 90 to 150 lb. the treatment differences are strongly significant, the value of  $z$  exceeding that required at the 1 per cent level of probability. The litter and heavy-light differences for this period are not significant. For the last period (150–200 lb.) the treatment and heavy-light differences are not significant, but there is a suggestion of significant litter differences, pens IV and V falling well below pens I, II and III.

It appears, therefore, that the moderate degree of significance attached to the results over the whole period is due to the minor differences that exist up to 90 lb. and from 150–200 lb. being included in the general

average. Only in the middle period (90–150 lb.) is there any marked evidence of differences in efficiency of food conversion between the rations. So far as these go, they point to the normal protein diet as being the most efficient, while we have to add to this information the fact that in this trial the litters in pens IV and V have proved the most economical feeders at the later stages of growth. An explanation of this last fact is difficult to adduce, unless it be a litter characteristic, for the two litters in question behaved very differently during the course of the experiment. *Pen IV started at a very high initial weight and made the greatest live-weight gain of all pens*, while pen V began well below average, and made only average live-weight gain.

The differences in efficiency of food conversion can be brought out in another way. We may, in fact, study the regression of live-weight gain ( $y$ ) on meal consumed ( $m$ ). The regression equation is

$$Y = 293.4 + 0.674 (m - 499).$$

The coefficient 0.674 is strongly significant, and corresponds to a correlation coefficient between the variates of +0.863. Adjusting the live-weight gains for constant total amount of meal consumed over the three treatments we get the following results, together with the earlier unadjusted results:

Live-weight gain in $\frac{1}{2}$ lb.	A	B	C	Mean
Unadjusted for meal consumed	302.0	292.9	285.3	293.4
Adjusted for meal consumed	299.4	291.6	289.2	293.4
Previous line in percentage	102.0	99.4	98.6	100.0

The drop from A to C in live-weight gain for the same amount of meal consumed is 3.4 per cent, with a standard error of 1.5. This drop is significant, as appears from the covariance analysis. Thus a falling off in live-weight gain with high protein feeding has been demonstrated even when the figures have been adjusted to what they might be expected to be had the same total amount of meal been consumed in all three groups over the 16-week period considered.

The analysis of the results for the group-fed pigs showed no significant influence of sex on the rate of L.W.I. The figures for the individually-fed pigs, however, are more suitable for investigation of sex influence, since in each of the five pens were six pigs from a given litter, a gilt and a brother hog being kept on each of the feeding treatments A, B and C. If the L.W.I. figures for the 16 weeks of the comparison be examined for differences relating to sex, we find, if the heavy-light comparison be ignored, that there is no significant difference between the averages of

the mean daily L.W.I. for hogs and gilts (hogs, 1.30 lb.; gilts, 1.32; s.e.=0.02). On the other hand, if we consider the data for lb. meal per lb. L.W.I. over the whole period of feeding up to 200 lb. L.W., we find a strongly significant sex difference, the gilts showing a greater efficiency of food conversion than the hogs. The average values are: hogs, 3.61 lb. meal per lb. L.W.I.; gilts, 3.45; mean, 3.53; s.e.=0.04.

Table XXIV. *Comparison between gilts and brother hogs*  
(48½ to 200 lb. L.W.)

Treat- ment	Litter	No. of pig	No. of days required	Total lb. L.W.I.	lb. L.W.I. per day	lb. meal per lb. L.W.I.
A	357	988	108	151	1.40	3.54
A	327	1013	121	151.5	1.25	3.88
A	342	999	109	151.5	1.39	3.56
A	208	970	105	152	1.45	3.34
A	437	949	102	151	1.48	3.28
B	357	991	120	151.5	1.26	3.87
B	327	1008	105	151	1.44	3.41
B	342	996	120	151.5	1.26	3.87
B	208	966	116	151	1.30	3.77
B	437	945	112	151.5	1.35	3.58
C	357	994	121	151.5	1.25	3.99
C	327	1007	118	151	1.28	3.85
C	342	1001	118	151	1.28	3.84
C	208	964	109	151.5	1.39	3.56
C	437	951	115	151.5	1.32	3.67
		Averages	113.3		1.34	3.67
Gilts						
A	357	993	109	151.5	1.39	3.44
A	327	1004	107	151.5	1.42	3.47
A	342	997	109	151.5	1.39	3.52
A	208	968	99	151.5	1.53	3.13
A	437	947	109	151.5	1.39	3.52
B	357	989	109	152	1.39	3.50
B	327	1010	109	151.5	1.39	3.54
B	342	998	113	151	1.34	3.66
B	208	967	110	151.5	1.38	3.54
B	437	946	106	151.5	1.43	3.42
C	357	987	110	151.5	1.38	3.53
C	327	1003	113	151	1.34	3.69
C	342	995	122	151	1.24	3.96
C	208	963	107	151.5	1.42	3.32
C	437	950	110	151.5	1.38	3.53
		Averages	109.5		1.39	3.52

In Table XXIV the results for the early days of the trial are excluded, and the data for the individually-fed pigs are given in every case over a period corresponding to a L.W.I. from 48½ lb. to 200 lb.

From the data in Table XXIV the significance of the drop in rate

of L.W.I. from treatment A to treatment C can again be demonstrated. The figures are:

Treatment ...	A	B	C	Mean	S.E.
Mean daily L.W.I. (lb.)	1.409	1.354	1.328	1.364	0.0186

The drop from A to C is 5.9 per cent with S.E. of 1.9.

Further, if we ignore the heavy-light comparison, the slight difference of rate of L.W.I. in favour of the gilts is now found to be significant.

	Hogs	Gilts	Mean	S.E.
Mean daily L.W.I. (lb.)	1.340	1.387	1.364	0.0152

### POST-SLAUGHTER RESULTS

#### *Experimental procedure following slaughter of pigs*

The dressed carcasses were weighed by the usual factory method, the hot carcass weight being thus ascertained to the nearest half-pound. The flares and kidneys were then removed and weighed. At this stage, in order to define the length of the "middle", the distance from the front rib near the backbone to the near edge of the *pubis symphysis* (aitch bone) was measured on both sides. The carcasses were then dispatched to the cooling room, where the backbones were chopped out and the fillets (psoas muscle) carefully excised. As with the flares and the kidneys, the fillets from both sides of the carcasses were weighed separately. They were transported to Cambridge later in the day, where, after they had been trimmed and weighed again, determinations of moisture and fat content were carried out. The iodine values of the petrol-ether-extracted fat were also determined by the method of Wijs.

The sides were allowed to cool for about 5 hours before the back fat and belly streak measurements were made. A standard pattern gauge, calibrated to read thickness in cm., was used for this purpose. Samples of back fat for analytical purposes were removed on the following morning from the gammon end of the sides. Storage of samples prior to analysis was effected in 100 per cent CO<sub>2</sub> at -5° C. Twenty-four hours later the weight of the chilled sides immediately before and after injecting with pickle was determined. The weights of the sides when freshly pickled and after maturing were also ascertained.

In order to secure more detailed information about the amount of lean and fat in the carcasses, one side from each pig was cut at a point in the back between the fourth and fifth ribs (counting from the gammon end) right through to the belly and a trace-drawing of the exposed surface made. The total area of the complete mid-back to belly rasher,



as well as the separate areas of lean and fat in the rasher, were then measured by means of a planimeter. A similar procedure was carried out on the surface exposed after cutting off the gammon from the sides. The significance of the information thus obtained will be made clear in the account that follows.

*Losses from fasting and from transport*

The pigs received their last meal on the morning before the day of slaughter, but an abundant supply of fresh water was available during the final fasting period. The journey by motor lorry to the bacon factory was over a distance of about 40 miles. In Table XXV are summarized the *average* losses from fasting and transport; the losses for the individual animals, however, are derivable from Tables XLVIII and XLIX.

Table XXV. *Losses from fasting and transport (June 1935)*

	Average final L.w. at farm lb.	Average fasted L.w. at farm lb.	Average loss from fasting lb.	Average L.w. at factory lb.	Average loss from transport lb.	Average total loss lb.
Individually-fed pigs						
Treatment A	206.0	204.0	2.0	201.8	2.2	4.2
" B	203.3	201.1	2.2	198.4	2.7	4.9
" C	204.5	201.6	2.9	198.6	3.0	5.9
Group-fed pigs						
Treatment A	204.7	201.3	3.4	197.3	4.0	7.4
" B	208.1	203.8	4.3	200.3	3.5	7.8
" C	204.6	201.3	3.3	198.0	3.3	6.6
Mean for all pigs	205.2	202.2	3.0	199.1	3.1	6.1

The individual total losses were extremely variable, ranging from as high as 12 lb. to as low as 1 lb., the average for the sixty pigs being 6.1 lb. This lack of consistency is to be expected, however, since the magnitude of the losses must be influenced greatly by the fullness of the stomach, intestines and bladder at the time of the final weighing before fasting. It is not desired, therefore, to draw any conclusions relating to the primary objects of the investigations from the figures in Table XXV; they are recorded simply on account of their practical interest to both curer and producer.

*Back fat and belly measurements*

In Table XXVI are recorded the measurements of the back fat and belly streak. Two measurements of the thickness of the back fat were made: (a) at the widest point over the shoulder; (b) at the thinnest point

near the last rib. Thickness of belly streak was defined by three measurements: (a) opposite the curve of the backbone; (b) opposite the junction of the fourth and fifth vertebrae from the curve; (c) at the lowest point near the ribs; i.e. at a distance below (b) roughly equal to the distance from (a) to (b). The factory gradings are also included in the table, these being based on the thickness of the shoulder fat and on measurement (b) of the belly streak.

A detailed study of the results in Table XXVI reveal occasional

Table XXVI. *Showing back fat and belly streak measurements and factory gradings*

No. and sex	Litter	Back fat			Belly streak			Grading		
		Max. cm.	Min. cm.	Mean cm.	(a) cm.	(b) cm.	(c) cm.	Mean cm.	Back fat	Belly streak
Individually-fed pigs										
Treatment A:										
G 993	357	5.05	2.55	3.80	3.45	4.15	5.30	4.30	B	A
H 988	"	5.05	3.15	4.10	2.50	3.45	5.20	3.72	B	A
G 1004	327	5.35	2.90	4.13	2.90	3.65	5.20	3.92	D	A
H 1013	"	5.70	3.00	4.35	3.75	3.75	5.15	4.22	D	B
G 997	342	5.45	2.45	3.95	3.30	3.50	4.40	3.73	C	A
H 999	"	5.45	2.70	4.08	2.65	2.80	4.60	3.35	C	C
G 968	208	5.40	2.95	4.18	3.05	3.85	4.38	3.76	B	A
H 970	"	5.55	3.00	4.28	2.70	3.70	4.55	3.65	D	B
G 947	437	5.35	2.60	3.98	3.35	4.25	4.50	4.03	D	A
H 949	"	5.45	2.85	4.15	2.80	3.65	4.50	3.65	D	C
Averages		5.38	2.82	4.10	3.05	3.68	4.78	3.84		
Treatment B:										
G 989	357	4.65	2.65	3.65	3.35	4.15	5.65	4.38	A	A
H 991	"	5.90	3.25	4.58	3.15	3.65	5.15	3.98	E	A
G 1010	327	4.95	2.50	3.73	2.70	3.30	4.75	3.58	C	A
H 1008	"	5.10	2.70	3.90	2.60	3.35	4.45	3.47	C	B
G 998	342	5.50	2.40	3.95	2.60	3.55	3.95	3.37	C	A
H 996	"	6.45	3.20	4.83	3.35	3.70	4.30	3.78	E	A
G 967	208	5.50	2.70	4.10	3.35	3.87	4.20	3.81	D	A
H 966	"	6.20	2.60	4.40	2.75	3.30	4.00	3.35	D	B
G 946	437	4.85	2.45	3.65	3.10	3.85	4.20	3.72	B	A
H 945	"	5.45	2.65	4.05	2.80	3.55	4.70	3.68	D	A
Averages		5.46	2.71	4.09	2.98	3.63	4.54	3.71		
Treatment C:										
G 987	357	5.50	2.80	4.15	3.70	4.35	5.10	4.38	C	A
H 994	"	5.75	2.95	4.35	3.25	3.90	4.85	4.00	D	A
G 1003	327	5.40	2.45	3.93	2.90	4.15	4.80	3.95	C	A
H 1007	"	5.25	2.70	3.98	2.50	3.30	4.50	3.43	D	B
G 995	342	6.10	2.65	4.38	3.00	4.00	4.85	3.95	D	A
H 1001	"	5.10	2.35	3.73	2.80	3.15	4.40	3.45	C	A
G 963	208	5.55	2.70	4.13	2.60	3.75	4.20	3.52	C	A
H 964	"	5.25	2.80	4.03	2.80	3.35	3.72	3.29	C	B
G 950	437	5.15	2.35	3.75	2.95	3.85	4.70	3.83	D	A
H 951	"	5.25	2.80	4.03	2.65	4.00	4.40	3.68	B	A
Averages		5.43	2.66	4.05	2.92	3.78	4.55	3.75		

Table XXVI (cont.)

No. and sex	Litter	Back fat			Belly streak			Grading		
		Max. cm.	Min. cm.	Mean cm.	(a) cm.	(b) cm.	(c) cm.	Mean cm.	Back fat	Belly streak
Group-fed pigs										
Treatment A:										
H 937	175	6.05	2.85	4.45	2.80	3.95	4.80	3.85	D	A
H 948	437	4.90	2.60	3.75	3.00	3.50	4.85	3.78	C	B
H 958	436	5.40	3.55	4.48	3.70	4.20	4.90	4.27	D	A
H 962	436	4.85	3.10	3.98	2.80	3.25	4.40	3.48	B	A
H 953	437	6.00	3.00	4.50	3.50	4.60	5.35	4.48	D	A
G 972	456	5.00	2.50	3.75	3.50	4.15	4.45	4.03	B	A
G 941	175	5.95	3.05	4.50	3.30	4.00	4.10	3.80	D	A
G 959	436	5.20	2.85	4.03	3.30	4.30	4.85	4.15	C	A
H 969	208	6.10	2.50	4.30	2.55	3.50	3.90	3.32	D	C
G 992	357	4.40	2.40	3.40	3.55	4.10	4.75	4.13	A	A
Averages		5.38	2.84	4.11	3.20	3.96	4.63	3.93		
Treatment B:										
H 940	175	5.25	2.70	3.98	3.05	3.63	4.45	3.71	C	B
H 943	437	5.45	2.75	4.10	3.45	4.30	5.15	4.30	D	A
H 960	436	5.10	2.90	4.00	4.15	4.60	4.95	4.57	C	A
H 955	436	5.70	3.90	4.80	3.90	4.55	5.50	4.65	D	A
G 954	437	5.25	2.85	4.05	2.95	4.10	4.20	3.75	B	A
H 971	456	4.95	2.80	3.88	3.25	3.48	4.55	3.76	C	B
G 965	208	5.40	3.00	4.20	2.45	3.35	3.85	3.22	D	A
G 986	357	6.20	3.35	4.78	3.55	4.60	5.20	4.45	D	A
G 1009	327	5.35	2.85	4.10	3.45	4.20	5.40	4.35	D	A
H 1000	342	4.95	2.45	3.70	2.55	3.35	5.00	3.63	B	B
Averages		5.36	2.96	4.16	3.28	4.02	4.82	4.04		
Treatment C:										
H 942	175	5.45	2.75	4.10	3.50	3.95	4.20	3.88	D	A
H 952	437	5.45	2.60	4.03	3.05	4.10	4.60	3.92	D	A
H 956	436	5.10	2.55	3.83	3.60	3.80	4.75	4.05	C	A
H 957	436	4.85	2.95	3.90	3.70	3.60	5.05	4.12	A	A
G 944	437	4.15	1.75	2.95	2.30	3.45	4.20	3.32	A	B
H 973	456	6.00	3.15	4.58	2.90	3.90	4.50	3.77	D	A
G 938	175	5.05	2.60	3.83	2.95	3.65	3.85	3.48	C	A
G 961	436	4.55	2.70	3.63	3.45	3.90	4.55	3.97	A	A
H 990	357	5.35	3.10	4.23	3.80	4.25	5.25	4.43	C	A
G 1005	327	4.95	2.55	3.75	2.85	3.75	4.95	3.85	C	A
Averages		5.09	2.67	3.88	3.21	3.84	4.59	3.88		

discrepancies between the factory gradings and the independent measurements made by the writers. This is perhaps to be expected, since the grading by the factory authority was carried out at 4 p.m. mainly on one side only, whereas the writers' measurements were made some 4 hours later and are the averages of measurements on both sides. In most cases the corresponding measurements on the two sides showed good agreement. There were, however, exceptional instances when this was not so, owing to unsymmetrical cutting or to natural lack of symmetry in the carcass. The disparities were observed more particularly in the case of the belly

measurements, differences ranging up to as much as 0.5 cm. between corresponding measurements on the two sides being occasionally noted. In the account that follows the conclusions are based on the average measurements on the two sides and not on the factory gradings.

*Influence of feeding treatments on back fat and belly streak*

The essential averages for the inquiry are summarized in Table XXVII.

Table XXVII. *Average back fat and belly streak measurements assembled according to treatment*

Treatment	Back fat			Belly streak			
	Max. cm.	Min. cm.	Mean cm.	(a) cm.	(b) cm.	(c) cm.	Mean cm.
Individually-fed pigs							
A (10 pigs)	5.38	2.82	4.10	3.05	3.68	4.78	3.84
B (10 pigs)	5.46	2.71	4.09	2.98	3.63	4.54	3.71
C (10 pigs)	5.43	2.66	4.05	2.92	3.78	4.55	3.75
S.E.			±0.072				±0.069
Group-fed pigs							
A (10 pigs)	5.38	2.84	4.11	3.20	3.96	4.63	3.93
B (10 pigs)	5.36	2.96	4.16	3.28	4.02	4.82	4.04
C (10 pigs)	5.09	2.67	3.88	3.21	3.84	4.59	3.88

It will readily be seen from the results in Table XXVII that the differences arising from treatment are very slight compared with the range of variation among the individuals within any one treatment (see Table XXVI). Statistical analysis of the results for the individually-fed pigs show that these small differences are not significant, and it may therefore be concluded that *the extremely wide variations in feeding treatments, involving an increase in the percentage of protein-rich food in the diet from normal to abnormally high levels, have been without significant effect on the thickness of back fat or belly streak.*

*Influence of sex on back fat and belly streak measurements*

The figures relevant to this question are given in Table XXVIII.

It has already been pointed out that the results for the individually-fed pigs should give the most reliable information about the influence of sex, since every gilt is paired off with a brother hog, and the lay-out is such as to reduce to a minimum possible disturbances from litter differences. That the unequal distribution between the sexes of the "heavies" and "lights" (see Table V) did not influence the results was

shown by a statistical examination of the figures for back fat and belly streak in relation to initial weight. No correlation was found, so that it may be concluded that sex differences in the measurements were undisturbed by variations in initial weight.

Table XXVIII. *Average back fat and belly streak measurements assembled to show influence of sex*

Treatment	No. of pigs in experiment		Mean back fat		Mean belly streak	
	Hogs	Gilts	Hogs	Gilts	Hogs	Gilts
			cm.	cm.	cm.	cm.
Individually-fed pigs						
A	5	5	4.19	4.01	3.72	3.95
B	5	5	4.35	3.82	3.65	3.77
C	5	5	4.02	4.07	3.57	3.93
	Averages		4.19	3.97	3.65	3.88
Group-fed pigs						
A	6	4	4.24	3.92	3.86	4.03
B	6	4	4.08	4.28	4.10	3.94
C	6	4	4.11	3.54	4.03	3.66
	Averages		4.14	3.91	4.00	3.88

The essential data for the individually-fed pigs are:

	Average of mean back fat measurements	Average of mean belly streak measurements
Hogs	4.19 cm.	3.65 cm.
Gilts	3.97 "	3.88 "
S.E.	$\pm 0.06$	$\pm 0.06$

The statistical analysis demonstrates that as a whole *the gilts have graded better than the hogs*, the differences between the averages of the mean back fat and belly streak measurements being significant. This conclusion receives confirmation from an analysis of the factory gradings. A more detailed inspection of the results, however, shows that the distinction in respect of back fat between the hogs and gilts to have been weaker than might be thought from the foregoing considerations, since only on treatment B was the difference in mean thickness of back fat statistically significant, the figures being: hogs, 4.35 cm.; gilts, 3.82 cm.; S.E. =  $\pm 0.10$ . On both treatments A and C the differences in back fat between gilts and hogs were not significant, and although out of ten pairs on these treatments there were eight cases in which the gilts had a thinner mean back fat than the brother hogs, it is clear that the differences were too small to be significant.

The data in the case of the group-fed pigs must be regarded as unsuitable for investigating the influence of sex, since it was impossible in

any one group to pair off every gilt with a brother hog. The influence of the sex factor was thus disturbed by litter differences, the magnitude of which in the case of the individually-fed pigs may be gauged from the following averages:

Litter	...	357	327	342	208	437	S.E.
Average of mean back fat data (cm.)		4.11	4.00	4.15	4.19	3.94	} $\pm 0.09$
Average of mean belly streak data (cm.)		4.13	3.76	3.61	3.56	3.77	

Litter differences in respect of back fat measurements were not significant, showing that in the present experiment the different litters in the individual-feeding lay-out display a good degree of uniformity from this standpoint. In regard to belly streak measurements, the differences between the average values for litters 327, 342, 208 and 437 were not significant. The average value for litter 357, however, was significantly higher, the individuals in this litter having a strong tendency to produce a thicker belly streak than the pigs in the other litters.

The group-fed pigs were not all drawn from the same litters as were used in the individual-feeding lay-out, so that the statements in the foregoing paragraph cannot be taken as applying in this case. It will be seen from Table XXVIII that the evidence from the group-feeding trial on the influence of sex on back fat and belly streak is of an inconsistent nature, permitting no trustworthy conclusions to be drawn. It is clear, therefore, that all discussions relative to the influence of sex in any connexion should be restricted solely to the results of the individual-feeding trial.

#### *Thickness of shoulder fat as a basis of grading*

A question of considerable interest in connexion with factory grading is whether the single measurement of the shoulder fat gives a reliable criterion of the general fatness of the pig along the whole length of the back. In Table XXIX are summarized, for the purpose of this inquiry, the shoulder fat and minimum back fat measurements of the pigs showing (a) the thickest and (b) the thinnest shoulder fat.

It will readily be noted that although on the average of a number of instances the shoulder fat tends to run roughly parallel with the minimum back fat, a consideration of the results for the individuals reveals striking exceptions. One example only need be quoted, that of A 993 and A 988, a hog and a gilt from the same litter. The measurements at the shoulder in these cases are equal, whereas the minimum back fat thicknesses are widely different.

The average figures suggest strongly that with pigs of thick shoulder

fat, the back fat tends to display a greater proportionate running-off in thickness along the length of the back than is the case with pigs of thinner shoulder fat. This implies that grading on the single basis of shoulder fat may impose a penalty on pigs of thick shoulder fat and that such pigs would come closer in the grades to pigs of thinner shoulder fat if the minimum back fat measurement were taken as the criterion.

Table XXIX. *Shoulder fat and minimum back fat measurements*

Individually-fed pigs			Group-fed pigs		
Treatment and no.	Shoulder fat cm.	Minimum back fat cm.	Treatment and no.	Shoulder fat cm.	Minimum back fat cm.
B 996	6.45	3.20	B 986	6.20	3.35
B 966	6.20	2.60	A 969	6.10	2.50
C 995	6.10	2.65	A 937	6.05	2.85
B 991	5.90	3.25	A 953	6.00	3.00
C 994	5.75	2.95	C 973	6.00	3.15
Averages	6.08	2.93		6.07	2.97
B 989	4.65	2.65	C 944	4.15	1.75
B 946	4.85	2.45	A 992	4.40	2.40
B 1010	4.95	2.50	C 961	4.55	2.70
A 993	5.05	2.55	A 962	4.85	3.10
A 988	5.05	3.15	C 957	4.85	2.95
Averages	4.91	2.66		4.56	2.58

The index employed for comparative purposes in the present investigation was the average of the maximum and minimum measurements. There is, however, a very strong correlation between this average measurement and the thickness of the shoulder fat ( $r=0.93$ ) arising partly from the fact that the average value is "weighted" by the shoulder fat measurement. It would appear, therefore, that the back fat comparisons among the pigs under experiment gained little in precision by relying on the mean of the two measurements instead of basing the comparisons on the shoulder fat alone.

Whether the shoulder fat affords a really reliable basis for grading can only be decided by working out the correlation between the shoulder fat and the mean thickness of back fat arrived at by averaging a large number of measurements at regular intervals along the back of the pig. Attention has been devoted to this aspect of the question in recent work, and an account of the findings will be given in a forthcoming publication.

#### *Grading of belly streak*

The factory grader measures the thickness of the belly streak at a point opposite the junction of fourth and fifth vertebrae from the curve. The question arises as to whether this single measurement affords a

reliable criterion of the average thickness of the belly, and data have been assembled in Table XXX to test this point. The figures relate to the pigs having (a) the thickest and (b) the thinnest belly streak at the point of the factory measurement. The values for mean belly thickness are the averages of three measurements (see Table XXVI).

Table XXX. *Grading measurements of belly streak compared with mean belly thickness*

Individually-fed pigs			Group-fed pigs		
Treatment and no.	Belly streak at juncture of vertebrae 4 and 5	Mean belly thickness	Treatment and no.	Belly streak at juncture of vertebrae 4 and 5	Mean belly thickness
	cm.			cm.	
C 987	4.35	4.38	A 953	4.60	4.48
A 947	4.25	4.03	B 960	4.60	4.57
A 993	4.15	4.30	B 986	4.60	4.45
B 989	4.15	4.38	B 955	4.55	4.65
C 1003	4.15	3.95	B 943	4.30	4.30
Averages	4.21	4.21		4.53	4.49
A 999	2.80	3.35	A 962	3.25	3.48
C 1001	3.15	3.45	B 965	3.35	3.22
C 1007	3.30	3.43	B 1000	3.35	3.63
B 1010	3.30	3.58	C 944	3.45	3.32
B 966	3.30	3.35	B 971	3.48	3.76
Averages	3.17	3.43		3.38	3.48

Although Table XXX shows that the values for mean belly thickness do not run consistently in the same order as the belly thickness at the point of factory measurement, it is clear that the latter, so far as the present experimental pigs are concerned, is a fairer index of general belly thickness than the shoulder fat measurement is of general fatness along the back. Little objection may be urged against the single measurement in so far as an index of thickness is desired. It may be argued, however, that the factory grading takes no account of the quality of the belly streak, and that a belly graded B on account of thinness may frequently be much leaner than a thicker belly securing grade A. On the other hand, a belly that is thick by virtue of fatness is liable to carry a penalty owing to thickness of fat over the shoulder, whereas thinness of belly accompanied by a high proportion of lean muscle in the streak is likely to escape this penalty.

*Weaning weight and rate of L.W.I. in relation to grading results*

It is sometimes claimed that a pig which has grown well during the suckling period and attained a good live weight by the date of weaning at 8 weeks (i.e. has made good progress at the time of its greatest capacity



for transforming food protein into body protein) will give a leaner carcass at 200 lb. L.W. than a pig that has made poor progress during suckling. The superiority claimed for the well-grown weaner is said to be emphasized if during the period from weaning to slaughter it progresses on the slow rather than on the quick side. On the other hand, it is claimed that the light weaner making quick growth from weaning to slaughter is likely to yield the fattest type of carcass.

The weaning weights of the individually-fed pigs varied from 20 to 39 lb. and the initial weights from 28½ to 50½ lb. Statistical analysis, however, fails to reveal any significant correlation between back fat or belly streak and weaning or initial weight. The factor of weaning weight has had no significant effect on the back fat and belly streak of the pigs in the present trial.

The results for the group-fed pigs are more suitable than those for the individually-fed animals for inquiring into the influence of rate of L.W.I. on the grading results because of the greater range of variation in the number of days required by the animals to reach slaughter weight. This was probably due to the impossibility of controlling the amount of food consumed by the individual members of the groups, so that certain of the pigs may have failed consistently to secure their fair share. The data essential to the inquiry are shown in Table XXXI.

Table XXXI. *Data for group-fed pigs illustrating relation of grading results to weaning weight and rate of L.W.I.*

Weaning weight lb.	48½–200 lb. L.W.		Mean back fat cm.	Mean belly streak cm.
	Days required	Mean L.W.I. per day (lb.)		
(1) Heavy weaners with slow L.W.I. (average of 5 pigs):				
40.0	129	1.17	3.89	4.02
(2) Heavy weaners with rapid L.W.I. (average of 6 pigs):				
36.3	109	1.39	4.11	3.93
(3) Light weaners with slow L.W.I. (average of 5 pigs):				
26.6	126	1.20	3.79	3.95
(4) Light weaners with rapid L.W.I. (average of 4 pigs):				
26.4	111	1.37	4.10	3.96

Although the data in Table XXXI reveal a tendency for the slower-growing animals to have on an average a slightly thinner back fat than the quicker-growing pigs, the differences between the means are so small compared with the range of individual variation that they are without significance. The evidence from a consideration of the individual cases

fails to point the way to any consistent conclusion on this point. It will further be noted that there are no significant differences in respect of the average belly measurements among the four divisions of the group-fed pigs. The results also confirm the finding that weaning weight has not influenced thickness of back fat or belly streak, since if rate of L.W.I. be ignored, the averages of the mean back fat measurements for the heavy and light weaners are 4.00 and 3.95 cm. respectively, while the corresponding averages for mean belly streak are 3.97 and 3.95 cm.

It should be emphasized that the foregoing conclusions do not preclude the possibility that a lengthening of the period of growth by deliberately restricting the food allowance might not be a means of producing leaner carcasses. Obviously the present experiment was not designed to test this distinct aspect of the question.

*Grading measurements in relation to length of pig*

It is frequently stated that, given pigs of equal live weight, the longer pig is likely to be the leaner one. Whilst this is most probably the case when the short pork breeds are compared with the long and slowly-maturing bacon breeds, it is not quite clear whether the influence of the length factor is significant when pigs of the same breed and strain are compared. The criterion of length in the present investigation was the distance from the front rib to the aitch bone (*pubis symphysis*), the measurements for all the pigs being given in Tables XLVIII and XLIX. The averages for the shorter (75.76.5 cm.) and the longer pigs (80.3-82.8 cm.) were:

	Fasted L.W. lb.	Length cm.	Mean back fat cm.	Mean belly streak cm.
Averages for 13 short pigs	199.6	75.9	4.25	3.99
Range of variation		75.0-76.5	3.73-4.83	3.35-4.65
Averages for 9 long pigs	204.8	81.0	4.11	3.73
Range of variation		80.3-82.8	3.75-4.50	3.29-4.05

It will be seen that, on an average, the longer pigs had a slightly thinner back fat and belly streak than the shorter pigs, but the differences between the means are so small compared with the range of variation among the individuals that they must be regarded as unimportant. It may be that the failure of the data to reveal any significant relation between length and grading measurements is due to the small range of variation (75.0-82.8 cm.) in the length of the "middle" among the sixty animals under experiment. The material may therefore have been unsuitable for enabling conclusions on this question to be made.

Table XXXII. Details of measurements on characteristic rashers (individually-fed pigs)

No. and sex	Litter	"Eye" muscle in mid-back rasher										Complete rasher							
		Gannon rasher				Belly rasher				Mid-back rasher			Space within line of connective tissue		"Eye" muscle		Back fat opposite "eye" cm.		
		Total area sq. cm.	% lean	% fat	% bone	Total area sq. cm.	% lean	% fat	% bone	Total area sq. cm.	% occupied by Muscle		% area Fat	Length cm.	Depth cm.	Total area sq. cm.		% fat	
Treatment A																			
G 993	357	236-1	44-7	86-6	27-9	70-4	1-7	122-0	29-1	69-8	1-1	29-6	88-5	11-5	8-3	4-6	2-9	210-6	70-0
H 998		240-5	45-5	83-5	32-4	64-6	1-5	127-7	29-6	69-4	1-0	40-0	73-0	27-0	8-7	6-1	2-7	221-9	67-4
G 1004	327	257-6	57-7	89-5	32-4	66-7	0-9	133-1	33-5	65-5	1-0	40-3	73-0	27-0	8-0	6-2	2-9	226-6	69-0
H 1013		250-9	43-8	82-1	30-8	68-2	1-0	122-9	31-7	77-5	0-8	28-0	74-6	25-4	7-0	4-2	2-9	205-0	73-6
G 997	342	231-9	59-5	88-2	32-4	65-3	2-3	117-1	32-3	66-0	1-7	32-5	81-8	18-2	8-5	4-5	2-9	205-3	63-7
H 999		241-6	57-7	79-5	37-1	60-4	2-5	122-4	31-7	67-1	1-2	34-5	84-6	15-4	8-6	5-1	2-4	201-9	64-4
G 998	266	283-4	64-5	90-7	37-4	62-3	0-3	113-0	48-1	51-4	0-5	36-1	95-8	4-3	9-0	6-4	3-4	203-7	56-2
H 996		232-7	55-0	82-3	32-9	62-9	1-6	110-1	28-7	70-4	0-9	28-5	87-7	12-3	7-8	5-0	3-2	193-4	68-8
G 947	437	262-9	59-2	94-6	33-9	64-0	1-2	101-2	35-7	63-6	0-7	34-9	81-7	18-3	9-0	5-0	3-2	195-6	64-2
H 949	"	219-5	59-5	86-0	34-4	63-5	2-1	118-4	31-4	67-3	1-0	36-8	81-8	16-2	7-5	6-7	3-2	204-4	65-9
Averages		245-6	54-8	89-0	33-7	64-8	1-5	118-8	32-2	66-8	1-0	34-1	83-7	16-3	8-3	5-4	2-9	206-8	66-0
Treatment B																			
G 999	357	271-0	56-6	80-6	34-5	64-4	1-1	118-1	39-5	59-1	1-4	30-7	100-0	0-0	7-5	6-0	2-5	186-7	61-2
H 991		266-3	51-4	95-1	22-3	75-7	2-0	95-4	36-3	62-9	0-8	34-6	78-3	21-7	9-0	5-5	3-1	190-5	69-3
G 1010	337	245-6	63-3	86-7	41-1	57-7	1-2	127-8	37-6	61-9	0-5	42-9	87-6	12-4	10-0	5-3	2-5	214-5	60-2
H 1006		236-9	43-7	83-9	26-9	62-0	1-5	106-1	30-9	63-4	0-8	39-1	87-2	12-8	8-5	5-4	3-0	217-6	62-8
G 996	342	231-4	49-6	93-6	20-0	78-5	1-5	132-0	22-8	76-4	0-8	30-8	77-3	22-7	8-2	4-4	4-0	231-6	77-2
H 997	206	272-8	54-1	93-8	33-9	65-0	1-1	138-0	34-3	64-6	1-1	39-0	85-6	14-4	8-0	6-0	3-9	225-8	64-7
G 946	457	215-4	55-7	91-1	26-5	71-9	1-6	115-6	26-2	72-4	1-4	30-0	78-3	21-7	9-2	5-5	3-2	206-7	72-2
H 945	"	229-7	61-1	76-3	37-2	59-4	3-4	111-3	40-3	58-1	1-6	35-0	86-9	13-1	8-0	5-5	2-2	187-6	58-6
Averages		276-1	55-6	92-2	35-4	63-0	1-6	117-0	29-6	69-2	1-2	37-9	89-9	30-1	7-8	4-8	2-4	209-2	66-4
Treatment C																			
G 987	357	231-9	59-2	104-7	34-4	63-5	3-1	125-9	31-7	67-0	1-3	34-4	75-3	24-7	6-5	5-5	3-2	230-6	64-9
H 984		271-5	59-6	93-6	23-0	68-9	2-1	150-5	47-1	71-7	1-2	37-6	83-0	17-0	7-5	6-6	3-9	244-1	70-6
G 1003	327	244-5	55-5	77-4	40-9	52-7	0-4	119-5	42-2	56-1	1-7	45-3	89-6	10-4	9-3	5-7	2-2	196-9	54-7
H 1007		215-9	50-2	80-9	28-8	70-1	2-0	117-6	33-1	66-2	0-7	34-2	87-7	12-3	8-7	5-1	2-6	188-7	69-6
G 986	342	230-4	40-2	75-2	27-0	67-4	1-6	101-8	30-9	67-4	1-7	31-7	87-7	15-3	8-6	5-0	2-6	188-7	69-6
H 1001		234-8	43-1	89-9	26-9	67-4	1-7	114-9	26-1	72-5	1-4	27-7	80-9	19-1	7-5	4-8	2-8	190-1	72-1
G 983	206	232-1	47-1	86-5	27-0	61-1	1-0	127-5	38-5	61-0	0-5	39-9	90-2	9-8	8-5	5-7	2-3	210-0	61-1
H 984		216-6	54-0	70-7	34-5	63-7	1-8	117-2	38-0	67-5	1-1	32-0	86-6	13-4	7-5	5-5	2-2	187-9	66-0
G 980	457	235-6	58-6	74-8	36-4	62-2	1-6	102-7	36-0	61-4	0-6	36-8	85-1	14-9	8-8	5-0	1-9	177-5	61-7
H 951	"	224-3	53-7	96-1	31-0	67-0	2-0	119-2	34-7	63-9	1-4	31-0	94-2	5-8	7-5	6-1	2-8	214-3	65-3
Averages		241-7	54-8	83-2	34-4	64-0	1-6	120-3	33-3	65-5	1-2	35-0	85-7	14-3	8-1	5-4	2-7	203-5	66-0



*Further criteria of leanness and fatness of carcasses*

It has already been explained how measurements were made of the size of, and the percentages of lean and fat in, certain typical rashers with a view to obtaining a further insight into the state of leanness or fatness of the different carcasses. In the case of every pig the following rashers were submitted to examination in this way: (1) the surface exposed in the side after removal of the gammon (referred to as the gammon rasher); (2) the surface exposed by cutting at a point in the back between the fourth and fifth ribs (counting from the gammon end) right through to the belly (referred to as the complete rasher). The trace-drawing of the complete rasher was divided into two portions of equal length, the portion containing the "eye" muscle being referred to as the mid-back rasher and the other portion as the belly streak rasher. The object of the separate study of the belly streak rasher was to take account of the possibility that high-protein feeding might result in the production of a leaner belly.

A large mass of comparative information relating to the fatness or leanness of the carcasses was secured by this procedure. The data are recorded in Tables XXXII and XXXIII.

*Size and leanness of gammon rasher in relation to feeding treatment*

The results in Table XXXIV for the individually-fed pigs demonstrate an *absence of influence of feeding treatment on the size and leanness of the gammon rasher*. The close agreement of the means is very noteworthy in view of the wide range of individual variation in the case of pigs on any one feeding treatment (see Table XXXII), a finding that points to the suitability of the individual-feeding lay-out for testing questions of this nature. It is interesting to note that the group-feeding data substantially confirm the foregoing conclusion. Just as group-

Table XXXIV. *Showing influence of feeding treatment on size and leanness of gammon rasher (treatment averages)*

Treatment	Individually-fed pigs				Group-fed pigs			
	Mean fasted L.W. at slaughter lb.	Area of rasher sq. cm.	% lean	% fat	Mean fasted L.W. at slaughter lb.	Area of rasher sq. cm.	% lean	% fat
A	204.0	245.6	54.8	45.2	201.3	235.1	50.3	49.7
B	201.0	252.5	54.9	45.1	203.8	250.5	56.3	43.7
C	201.6	241.7	54.8	45.2	201.3	243.7	56.3	43.7
	S.E.	±6.5	±1.8	±1.8				

feeding yielded results in respect of L.W.I. roughly confirming the more precise findings of the individual-feeding trial, so here again the results from the two techniques are in similar harmony. As with the results for L.W.I., however, the standard errors for the group-feeding data are of necessity much larger than for the individual-feeding results.

*Size and leanness of gammon rasher in relation to sex*

The data for the individually-fed pigs in Table XXXV suggest that the gilts, on an average, tend to produce a slightly leaner gammon rasher than the hogs. Statistical analysis, however, reveals that the differences, both in respect of size and leanness of the rasher, are not significant.

Table XXXV. *Showing influence of sex on size and leanness of gammon rasher*

Treatment	Gilts		Hogs	
	Area of rasher sq. cm.	% lean	Area of rasher sq. cm.	% lean
A (average of 5 pigs)	254.2	56.7	237.0	52.9
B (       "       )	251.2	55.8	253.8	54.1
C (       "       )	248.7	56.5	234.6	53.0
			S.E. = $\pm 1.5$	
General averages	251.4	56.3	241.8	53.3
	S.E. = $\pm 5.3$			

*Size and leanness of gammon rasher in relation to litter*

The averages of the gammon rasher measurements for the pigs in the five litters constituting the individual-feeding lay-out are shown in Table XXXVI, from which it will be seen that litter had been almost without significant influence on both the size and leanness of the rasher. It would appear, however, that litter 342 gave a significantly fatter rasher than litter 437. There is a very considerable range of variation in both respects among the individuals in any one litter, pointing to the strong influence of the factor of idiosyncrasy (see Table XXXII). The disturbing effect of this factor, however, is taken into account in the computation of the standard error.

Table XXXVI. *Showing influence of litter on size and leanness of gammon rasher*

Litter	...	357	327	342	208	437	S.E.
Average area (sq. cm.)		251.2	249.1	243.0	245.3	244.4	$\pm 8.3$
Average % lean		53.1	55.5	50.6	56.8	58.2	$\pm 2.4$

*Size and leanness of belly streak rasher in relation to feeding treatment*

The average values for the pigs under the three treatments are given in Table XXXVII. It is not considered necessary to include the figures for the percentage of bone in the rasher, but these may be obtained, if desired, from Table XXXII. It will be concluded from the data in Table XXXVII that *the widely-differing feeding treatments have led to no significant differences in the size and leanness of the belly streak rasher*. The statistically-treated results of the individual-feeding lay-out are again strikingly confirmed by the results of the group-feeding trial.

Table XXXVII. *Showing influence of feeding treatment on size and leanness of belly streak rasher (treatment averages)*

Treat- ment	Individually-fed pigs				Group-fed pigs			
	Mean fasted L.W. at slaughter lb.	Area of rasher sq. cm.	% lean	% fat	Mean fasted L.W. at slaughter lb.	Area of rasher sq. cm.	% lean	% fat
A	204.0	88.0	33.7	64.8	201.3	88.7	32.7	65.2
B	201.1	88.4	31.3	67.0	203.8	86.5	32.2	66.0
C	201.6	83.2	34.4	64.0	201.3	83.2	32.9	65.6
	S.E.	±2.4	±1.3					

*Size and leanness of belly streak rasher in relation to sex*

The average results relating to the belly streak rasher (individual-feeding trial) are arranged in Table XXXVIII to bring out any possible differences arising from the influence of the sex factor. The main fact emerging from the figures is that, judged on the basis of the general averages, the gilts had significantly leaner bellies than the hogs. This effect of sex, however, appears to be shown only by the pigs under treatments B and C and not by those under treatment A. If, however, the amount of lean in the belly streak rasher be stated as an actual area, it is seen that even in the case of treatment A, the gilts on an average had a slightly larger area of lean (29.9 sq. cm.) than the hogs (29.2 sq. cm.).

A consideration of the treatment averages in Table XXXVIII suggests that the gilts tended to produce a leaner belly as the percentage of protein-rich food in the diet was increased, a behaviour not displayed by their brother hogs. This possible treatment effect, however, is not significant, since the S.E. of the means for the gilts under treatments A and C (32.8 and 36.9 per cent respectively) is  $\pm 1.88$ . It should also

be noted that the general averages demonstrate an absence of significant sex effect on the size of the rasher.

Table XXXVIII. *Showing influence of sex on size and leanness of belly streak rasher*

Treatment	Gilts		Hogs	
	Area of rasher sq. cm.	% lean	Area of rasher sq. cm.	% lean
A (average of 5 pigs)	91.1	32.8	84.8	34.5
B (       "       )	84.3	34.5	92.5	28.1
C (       "       )	85.3	36.9	81.2	31.8
			S.E. = $\pm 1.08$	
General averages	86.9	34.7	86.2	31.5
			S.E. = $\pm 1.98$	

*Size and leanness of belly streak rasher in relation to litter*

The averages given in Table XXXIX for the pigs in the five litters of the individual-feeding lay-out show that the effect of litter on the size of the belly streak rasher was not significant. There are, however, undoubtedly significant litter differences in respect of the leanness of the rasher, the pigs in litter 327 being, on an average, significantly leaner than the pigs in litters 357 and 342.

Table XXXIX. *Showing influence of litter on size and leanness of belly streak rasher*

Litter	...	357	327	342	208	437	S.E.
Average area (sq. cm.)		92.8	83.6	83.6	86.2	86.5	$\pm 3.14$
Average % lean		30.4	37.6	28.5	34.5	34.7	$\pm 1.71$

*Size and leanness of mid-back rasher in relation to feeding treatment*

The close agreement of the means in Table XL for the pigs under the three feeding treatments is very noteworthy. Statistical analysis of the results for the individually-fed pigs demonstrate that *differences in feeding treatment have been without influence on the size and leanness of the mid-back rasher*, a finding that is fully confirmed by the results of the group-feeding experiment. The general conclusion, therefore, may be drawn that *a far-reaching replacement of carbohydrate by protein in the rations of Large-White baconers has not led to the production of leaner carcasses, if the data for the three typical rashers from the back, belly and gammon, as well as the back-fat measurements, be taken as the criteria.*



Table XL. *Showing influence of feeding treatment on size and leanness of mid-back rasher (treatment averages)*

Treatment	Individually-fed pigs				Group-fed pigs			
	Mean fasted L.W. at slaughter lb.	Area of rasher sq. cm.	% lean	% fat	Mean fasted L.W. at slaughter lb.	Area of rasher sq. cm.	% lean	% fat
A	204.0	118.8	32.2	66.8	201.3	121.1	32.3	66.2
B	201.1	118.8	33.3	65.6	203.8	124.5	32.7	66.0
C	201.6	120.3	33.3	65.5	201.3	119.4	32.5	66.0
	S.E.	±3.83	±1.57					

*Size and leanness of mid-back rasher in relation to sex*

The general averages for the individually-fed pigs in Table XLI clearly demonstrate that the size of the mid-back rasher was not influenced significantly by sex. A decidedly significant conclusion is possible regarding the influence of sex on the leanness of the rasher, however, the gilts having a definitely leaner back rasher than the hogs. An analysis of the fifteen separate comparisons between gilts and brother hogs on the same feeding treatments (see Table XXXII) reveals the remarkably consistent nature of this finding.

Table XLI. *Showing influence of sex on size and leanness of mid-back rasher*

Treatment	Gilts		Hogs	
	Area of rasher sq. cm.	% lean	Area of rasher sq. cm.	% lean
A (average of 5 pigs)	117.8	35.7	120.3	28.6
B ( " )	119.1	36.5	118.6	30.1
C ( " )	116.7	36.3	123.9	30.5
			S.E. = ±1.28	
General averages	117.7	36.2	120.9	29.7
	S.E. = ±3.18			

*Size and leanness of mid-back rasher in relation to litter*

The litter averages in Table XLII display no significant differences in regard to size of rasher. It is interesting to note, however, that the means do not show the same trend as the corresponding means for the belly rasher, a fact which suggests a possible litter influence on conformation.

Although litter appears also to have had no significant influence on

the leanness of the back rasher, it may be pointed out that the series of litter averages for the percentage of lean in the mid-back rasher has features in common with the corresponding data for the gammon and belly streak rashers. In all cases, for example, litter 342 produced, on an average, the fattest rasher.

Table XLII. *Showing influence of litter on size and leanness of mid-back rasher*

Litter	...	357	327	342	208	437	S.E.
Average area (sq. cm.)		123.3	124.7	117.7	119.3	111.7	$\pm 4.95$
Average % lean		32.2	34.0	29.2	34.4	35.0	$\pm 2.03$

*Size of "eye" muscle in relation to feeding treatment*

The important part of the mid-back rasher from the consumer's standpoint is the lean "eye" muscle. Measurements of the area occupied by this muscle, as shown in the trace-drawing of the rasher, were made in every case, as well as of the extent of fat infiltration into the area within the line of connective tissue. The full details are recorded in Tables XXXII and XXXIII, whilst in Table XLIII are summarized the averages for the pigs under the three feeding treatments. It is clear from the statistical analysis of the figures for the individually-fed pigs that *the character of the feeding has been without significant influence on the dimensional characteristics of the "eye" muscle* as defined by the area within the line of connective tissue. The averages of the actual areas occupied by lean muscle for the individually-fed pigs under treatments A, B and C were 28.6, 29.3 and 30.1 sq. cm. respectively (s.e. =  $\pm 1.27$ ), results that again demonstrate an absence of significant influence of feeding treatment. These findings are amply confirmed by the results of the group-feeding trial.

It would appear that the size of the "eye" muscle is decided mainly by the factor of inheritance and is not greatly influenced by the character of the feeding, provided, of course, that the diet is not actually deficient in protein. Treatment C, furnishing an excessive supply of protein, neither prevented fat infiltration nor even affected significantly the extent to which this occurred. The protein fed in excess of normal growth requirements has manifestly not been utilized to increase the size of the "eye" muscle. This finding, however, does not rule out the possibility that a ration actually *deficient* in protein would lead to increased fat infiltration into the area within the line of connective tissue.

Table XLIII. *Showing influence of feeding treatment on "eye" muscle measurements (treatment averages)*

Treatment	Back fat opposite "eye" cm.	Individually-fed pigs						Group-fed pigs						Back fat opposite "eye" cm.
		Space within line of connective tissue				"Eye" muscle		"Eye" muscle		Space within line of connective tissue				
		Area sq. cm.	% area occupied by		Length cm.	Depth cm.	Length cm.	Depth cm.	Area sq. cm.	% area occupied by				
			Muscle	Fat						Muscle	Fat			
A	2.9	34.1	83.7	16.3	8.3	5.4	8.3	5.3	34.3	81.4	18.6	2.9		
B	2.8	35.1	83.1	16.9	8.4	5.1	8.3	5.3	35.5	82.6	17.4	2.9		
C	2.7	35.0	85.7	14.3	8.1	5.4	8.3	5.1	35.2	84.1	15.9	2.8		
S.E.		±1.36	±1.98											

*Size of "eye" muscle in relation to sex*

The data in Table XLIV for the individually-fed pigs are arranged to bring out the influence of sex on the size of the "eye" muscle. It will be noted that the gilts had, on the average, a significantly larger "eye" muscle than the hogs. Consistent with this is the finding that the "eye" muscle of the gilts averaged a significantly higher percentage of the area within the line of connective tissue than did that of the hogs, i.e. there was significantly less fat infiltration in the case of the gilts.

Table XLIV. *Showing influence of sex on size of "eye" muscle and extent of fat infiltration*

Treatment	Gilts			Hogs		
	"Eye" muscle			"Eye" muscle		
	Area sq. cm.	Depth cm.	% area occupied by muscle	Area sq. cm.	Depth cm.	% area occupied by muscle
A (average of 5 pigs)	30.2	5.3	—	26.9	5.4	—
B ( " " )	31.4	5.5	—	27.0	4.7	—
C ( " " )	32.1	5.4	—	28.1	5.4	—
	S.E. = ±1.04					
General averages	31.2	5.4	86.7	27.3	5.2	81.7
	S.E. = ±1.60					

*Size of "eye" muscle in relation to litter*

Statistical analysis of the litter averages in Table XLV reveals no significant effect of litter on the size of the "eye" muscle. It is nevertheless of interest to note that the pigs in litter 342, which on an average gave the smallest and thinnest "eye" muscle, also displayed a tendency to produce less lean and more fat than the pigs in the other litters by yielding, on an average, the fattest gammon, belly and mid-back rashers.

The data in respect of the depth and length of the "eye" muscle lead to contradictory conclusions. Owing to the varying shape of the "eye" muscles of the individual pigs, it is clear that measurements are essentially unreliable criteria of the size of the "eye" muscle, and in future work these measurements will not be made.

Table XLV. *Showing influence of litter on size of "eye" muscle*

Litter ...	357	327	342	208	437	S.E.
Average area (sq. cm.)	28.4	33.1	25.6	30.1	29.4	$\pm 1.64$
Average depth (cm.)	5.6	5.3	4.8	5.4	5.6	

*Correlation of weaning weight and rate of L.W.I. with size of "eye" muscle*

The hypothesis is sometimes put forward that the size of the "eye" muscle is determined by the progress of the young pig during the suckling period. A well-developed "eye" muscle at 200 lb. L.W., according to this theory, is to be associated with strong and vigorous growth in the pre-weaning stages. The results of this trial, however, do not support this hypothesis, the correlation between weaning weight and size of "eye" muscle being totally insignificant ( $r = -0.013$ ).

Neither is there sufficient evidence to warrant a significant correlation between the size of "eye" muscle and the L.W.I. over the 16 weeks of the feeding comparison to be postulated with certainty. Ignoring treatment,  $r = +0.412$ . This is barely significant, since for 5 per cent probability, the value should be 0.433. It would seem, however, that a moderate degree of relationship does exist, and that the animals making the biggest live-weight increases tend also to have the largest "eye" muscles.

*Size and fatness of complete rasher in relation to feeding treatment*

The results for the separate mid-back and belly rashers, obtained by halving the complete rasher from the length standpoint, may not be so suitable for elucidating the effect of treatment as the figures for the complete rasher, since the division of the latter into two portions is somewhat arbitrary in view of its varying shape from pig to pig. The average figures in Table XLVI for the pigs under the three treatments clearly substantiate the conclusion already arrived at, namely, that *the large progressive increases in the percentage of protein in treatments A, B and C have led to no corresponding increases in carcass leanness.*

Table XLVI. *Showing influence of feeding treatment on size and leanness of complete rasher (treatment averages)*

Treatment	Individually-fed pigs			Group-fed pigs		
	Mean fasted L.W. at slaughter lb.	Area of rasher sq. cm.	% fat	Mean fasted L.W. at slaughter lb.	Area of rasher sq. cm.	% fat
A	204.0	206.8	66.0	201.3	209.8	65.8
B	201.1	207.2	66.3	203.8	211.0	66.0
C	201.6	203.5	65.0	201.3	202.6	65.8

*Size and leanness of complete rasher in relation to sex*

The general averages in Table XLVII for the percentages of fat in the complete rasher show that the gilts on an average produced a significantly leaner rasher than the hogs. Reference to the detailed figures for the individually-fed pigs in Table XXXII demonstrates that this sex distinction was manifested with a high degree of consistency in the comparisons between gilts and brother hogs on the same feeding treatment. The difference between the general means for the size of the rasher is not significant.

Table XLVII. *Showing influence of sex on size and fatness of complete rasher*

Treatment	Gilts		Hogs	
	Area of rasher sq. cm.	% fat	Area of rasher sq. cm.	% fat
A (average of 5 pigs)	206.6	64.4	205.1	67.7
B ( " )	203.3	62.9	211.1	69.6
C ( " )	201.9	62.2	205.1	67.6
			S.E. = $\pm 1.1$	
General averages	203.9	63.2	207.1	68.3
	S.E. = $\pm 4.6$			

*Back-fat measurements as criteria of carcass fatness or leanness*

One of the most debatable points in connexion with the present grading system is whether the thickness of the shoulder fat is a reasonably reliable indication of the general condition of fatness in the pig. An endeavour has been made in these experiments to secure enlightenment on this question by making an elaborate investigation of typical rashers, and it will now be of interest to examine the relation of the results so obtained to the back-fat measurements. If the figures showing the

thickness of shoulder fat, mean back-fat thickness, percentages of fat in the gammon and complete rashers be tabulated for all the pigs in the order of descending mean back-fat thickness (see Tables XXVI, XXXII and XXXIII) it will be seen that there is, in general, a tendency for fatness in the rashers to run parallel with the mean thickness of back fat. The correspondence, however, is very imperfect, and unaccountable anomalies are met with. It is, therefore, only feasible to arrive at conclusions by statistical analysis of the figures.

It has already been shown that there is a very strong correlation between the mean back-fat and shoulder-fat measurements ( $r=0.93$ ), a finding suggesting that, for comparative purposes among the animals used in this experiment, these measurements were of about equal value as an index of thickness of fat along the back.

The correlation between mean back fat and the percentage of fat in the complete rasher is significant ( $r=0.45$ ), a finding that points to a general tendency for the fatness of the rasher to increase with increase of the mean thickness of back fat. A more detailed analysis of the results, however, reveals the need for caution in coming to any definite conclusion on the basis of the present data. In view of the distinctions, attributable to sex, that have been noted in respect of thickness of back fat and percentages of fat in the typical rashers, it is of interest to ascertain if the correlation between mean back-fat thickness and fatness in the complete rasher is still significant when the results for gilts and hogs are considered separately. Taking first the figures for the individually-fed pigs, the correlation remains significant for the hogs ( $r=0.65$ ) but becomes insignificant in the case of the gilts ( $r=0.25$ ). In the latter instance, therefore, the mean back-fat thickness could not be regarded as a satisfactory criterion of the fatness of the rasher. The correlation in the case of the group-fed hogs, probably as a consequence of a higher standard error, falls just short of actual significance ( $r=0.42$ ). No attempt was made to analyse from this standpoint the figures for the group-fed gilts, of which there were only twelve under experiment.

A similar uncertainty is encountered when the correlation between the thickness of the shoulder fat and the percentage of fat in the complete rasher is worked out. The correlation is significant for hogs and gilts together ( $r=0.56$ ), but when the sexes are considered separately, the correlation in the case of the hogs (individually-fed pigs) is still significant ( $r=0.72$ ) but insignificant for the gilts ( $r=0.34$ ).

No attempt will be made at this point to account for this distinction between the hogs and the gilts. Rather is it considered desirable to

accumulate sufficient evidence to demonstrate the reality of the finding before seeking explanations. On the basis of the present limited data, however, it would seem that the degree of statistical significance of the correlations is not particularly high, and that therefore the thickness of the shoulder fat or the average of the maximum and minimum thicknesses of back fat may not be entirely satisfactory criteria of the general fatness of the carcass. The writers will return to this question in a future publication, when a much more detailed examination of this aspect of the grading problem will be undertaken.

It may finally be noted that a significant correlation exists between the percentages of fat in the gammon and complete rashers ( $r=0.64$ ), but that the mean thickness of back fat and the fatness of the gammon rasher are not significantly correlated.

*Leanness of complete rasher in relation to length of pig*

The averages for the thirteen short pigs (75–76.5 cm.) and the nine long pigs (80.3–82.8 cm.) are as follows:

	Fasted L.W. lb.	Length cm.	% fat in complete rasher
Averages for 13 short pigs	199.6	75.9	69.5
Averages for 9 long pigs	204.8	81.0	63.8

The results suggest strongly that the longer pigs tended to produce the leaner rashers, a finding that is confirmed by statistical analysis, the correlation between the length of the “middle” (front rib to *pubis symphysis*) and the percentage of fat in the complete rasher being negative and significant ( $r = -0.47$ ).

#### FURTHER POST-SLAUGHTER RESULTS

In Tables XLVIII and XLIX are recorded the results of further measurements that were made immediately after the slaughter of the pigs or during the curing processes. The following points require to be emphasized: (1) the carcass weights are the so-called “hot” carcass weights, which were determined to the nearest half-pound. A deduction of 4 lb. for pigs in Class I is necessary, to allow for shrinkage on cooling, in order to arrive at the factory “dead weights”; (2) the figures for the kidneys and flares are the average weights of these from the two sides and should therefore be doubled if the total weights are required.

Table XLVIII. Showing further post-slaughter results (individually-fed pigs)

No. and sex	Litter	Final L.w. lb.	Fasted L.w. at farm lb.	Fasted L.w. at factory lb.	"Hot" carcass per-centage	Length of middle cm.	Average weight of flank g.	Average weight of kidney g.	Iodine value of back fat	Fillets			Weight of sides before curing oz.	Weight of gammon oz.	
										Total weight of both fillets g.	% water	% fat			
Treatment A															
G 983	357	204½	198	196	81.6	76.5	899	138.8	63.4	63.4	534	76.1	1.8	1979	250.5
H 988	"	208	207	207	82.4	77.3	831	114.5	62.6	63.1	558	76.8	2.1	2105	272.5
G 1004	327	202	199½	199	82.4	76.0	770	115.0	63.1	63.1	567	75.8	2.1	2025	255
H 1013	"	204	200½	198	82.8	76.0	1171	138.5	60.6	64.2	440	76.8	1.5	1933	238
G 997	342	201½	201	201	80.3	79.0	857	163.5	64.2	62.9	691	77.0	2.1	1948	240.5
H 999	"	205½	203	203	80.8	80.0	749	156.0	62.9	62.9	691	77.0	1.6	1970	246
G 968	208	213	208	208	82.9	81.0	736	124.5	62.9	61.8	478	76.1	1.9	2088	273.5
H 970	"	210	210	207	80.9	78.8	769	114.3	61.8	61.8	478	76.1	1.9	2026	234
G 947	437	204½	202	198	82.3	77.8	1024	102.5	61.8	60.0	76.0	76.0	1.9	2023	243.5
H 949	"	207	206	201	81.3	81.0	829	117.5	61.6	61.6	499	76.4	2.0	1975	234
Means		206.0	204.0	201.8	81.8	78.3	864	128.5	62.49	62.49	553.5	76.4	1.89	2007	248.8
Treatment B															
G 989	357	205½	202	198	83.3	78.3	736	134.0	63.6	63.6	569	76.1	1.5	2026	265
H 991	"	203	199½	196	83.2	75.8	1038	127.5	61.0	61.0	535	76.4	2.2	1986	225
G 1010	327	200	196	196	81.4	75.0	591	123.5	66.4	66.4	754	76.8	1.5	1956	248
H 1008	"	204	202	199	82.4	77.5	765	112.0	62.4	62.4	589	75.8	1.6	2005	252
G 998	342	204	204	201	81.6	79.3	935	135.0	64.9	62.9	523	77.1	1.7	1981	245.5
H 996	"	207½	203½	197	85.5	76.5	1144	128.5	61.6	61.6	469	76.8	2.0	2046	239
G 967	208	203	202	199	81.7	78.8	770	141.0	64.0	64.0	555	75.5	1.7	2005	249
H 966	"	200	199	198	81.1	76.5	809	114.5	65.3	65.3	496	76.4	2.4	1928	245
G 946	437	200½	199	197	81.0	78.5	789	127.0	63.3	63.3	606	76.1	1.6	1969	249.5
H 945	"	205½	204	203	80.8	78.3	822	122.0	60.1	60.1	531	76.4	1.8	1982	251.5
Means		203.3	201.1	198.4	82.2	77.6	840	126.5	63.26	63.26	562.7	76.3	1.80	1987	247.0
Treatment C															
G 987	357	203½	200	196	83.2	78.0	631	143.5	64.2	64.2	594	76.4	1.2	2025	253
H 994	"	210½	207	205	82.7	78.0	1043	139.0	62.3	62.3	767	76.8	2.1	2082	255.5
G 1003	327	204½	201	199	82.4	78.8	630	143.5	65.8	65.8	567	76.5	1.4	1978	251.5
H 1007	"	203	198	193	82.9	76.3	828	135.0	62.3	62.3	548	77.2	1.9	1950	220.5
G 995	342	203	201½	199	82.9	82.3	761	166.5	63.0	63.0	502	76.7	1.8	2002	241
H 1001	"	200½	200	198	80.1	77.8	889	160.5	63.5	63.5	668	77.2	2.3	1885	232.5
G 963	208	213	210	207	81.2	80.8	592	143.5	67.0	66.2	76.4	76.4	1.5	2063	249.5
H 964	"	204	202	197	80.2	80.3	736	131.5	62.9	62.9	486	76.1	2.6	1916	236
G 950	437	201	198	193	81.3	80.3	955	135.0	64.0	64.0	556	76.4	1.2	1923	232
H 951	"	202	199	199	80.0	77.0	717	140.5	63.7	63.7	477	76.8	1.9	1926	246.5
Means		204.5	201.6	198.6	81.7	79.0	778	143.9	63.87	63.87	582.7	76.6	1.79	1975	241.8



Table XLIX. Showing further post-slaughter results (group-fed pigs)

No. and sex	Litter	Final L.W. lb.	Fasted		"Hot" carcass per-centage	Length of middle cm.	Average weight flare g.	Average weight kidney g.	Iodine value of back fat	Total weight of both fillets g.	Filets		Weight of sides before curing oz.	Weight of gammon oz.
			L.W. lb. at farm	L.W. lb. at factory							% water	% fat		
Treatment A														
H 937	175	206	203	199	82.9	79.8	1028	115.0	62.6	464	76.6	1.5	70.9	1992
H 948	437	204	204	199	78.9	77.8	848	117.0	61.4	445	76.0	2.3	57.6	1921
H 958	436	206	200	196	84.4	75.0	1080	110.0	61.9	434	76.0	2.7	57.0	2028
H 962	436	201	197½	196	79.6	77.5	1054	108.0	61.6	592	76.5	2.2	63.9	1904
H 953	437	201	196	189	83.2	80.0	925	115.5	59.9	529	75.6	1.5	68.2	1960
G 972	456	213	206	206	80.6	78.8	716	105.5	66.5	799	76.5	1.8	68.3	2044
G 941	175	217	212	207	84.1	82.8	1083	130.8	66.2	618	75.7	1.4	61.7	2117
G 959	436	200	196½	191	83.8	76.3	821	99.0	61.5	531	76.9	1.5	64.9	1973
H 969	208	200	197½	192	79.2	79.0	834	119.0	65.3	387	76.1	2.2	58.8	1808
G 992	337	199	197	191	81.9	77.0	629	115.0	62.9	577	76.0	1.5	65.2	1943
Means		204.7	201.3	197.3	81.9	78.4	902	113.5	63.0	537.6	76.2	1.86	63.7	1969
Treatment B														
H 940	175	203	199	196	80.6	79.8	848	123.0	69.0	502	75.8	2.2	60.9	1888
H 943	437	206	203½	201	81.1	78.8	881	115.0	61.2	504	76.2	1.6	69.6	1988
H 960	436	201½	197½	192	83.6	76.0	1083	125.0	63.3	514	76.6	1.9	63.6	1909
H 965	436	206	203	201	83.8	75.5	1031	132.0	62.1	438	75.4	2.5	63.8	2065
G 964	437	209½	200	198	81.1	80.5	729	114.0	65.3	596	76.0	1.4	70.4	1950
H 971	456	199	199	196	79.9	78.5	740	115.0	64.9	483	76.4	2.1	60.1	1902
G 965	208	211	206	204	79.7	78.0	686	120.0	62.7	620	76.9	1.5	71.6	1983
G 986	337	235	230	223	83.9	79.8	1136	147.5	64.1	556	75.6	1.8	67.8	2323
G 1009	327	202½	198	196	82.9	79.0	803	132.0	64.4	585	76.6	1.3	68.1	1986
H 1000	342	208	202	196	83.4	78.0	770	125.0	63.4	737	77.0	1.8	67.5	1992
Means		208.1	203.8	200.3	82.0	78.4	871	124.9	64.0	553.5	76.3	1.81	66.3	2002
Treatment C														
H 942	175	201	197	192	83.1	76.0	1101	130.3	65.5	492	76.6	1.5	66.5	1913
H 952	437	203	198½	196	81.1	78.5	939	153.5	61.4	519	75.8	1.8	67.8	1930
H 966	436	203½	201	198	81.2	80.3	1265	141.0	62.3	460	76.8	1.7	64.7	1910
H 967	436	203	201	197	81.2	75.8	1064	143.0	63.3	504	76.5	1.9	62.2	1943
G 944	437	199½	196	191	78.8	79.5	536	132.0	66.0	551	76.8	1.2	79.2	1702
H 973	456	210½	205	204	83.3	78.3	764	146.8	62.1	538	75.7	2.4	58.8	2118
G 938	175	211	206	200	82.0	78.3	812	152.0	64.9	545	76.9	1.2	67.4	1996
G 961	436	202	199	199	81.4	79.0	940	133.0	61.2	674	76.5	1.8	66.1	1992
H 990	337	204	203	200	81.3	78.3	815	145.0	61.1	464	76.4	1.7	56.8	2020
G 1005	327	208½	206	203	81.5	78.3	822	150.5	60.6	573	76.5	1.3	64.7	2049
Means		204.6	201.3	198.0	81.5	78.2	882	142.7	62.8	532.0	76.4	1.65	65.4	1966
														243.1

*Length of pig in relation to feeding treatment, sex and litter*

The data essential to this inquiry are summarized in Table L and refer to the individually-fed pigs. The conclusion is to be drawn that *the feeding treatments, with their widely-differing protein supplies, have led to no significant differences in the length of the pigs* as measured from the front rib to the *pubis symphysis*, a finding that receives support from the results for the group-fed pigs (mean lengths for treatments A, B and C being 78.4, 78.4 and 78.2 cm. respectively).

It is also concluded that difference between the average lengths of the hogs and gilts is not statistically significant, although the figures suggest a tendency for the gilts to give a longer carcass than the hogs. This, of course, would be consistent with the finding that the gilts produce somewhat leaner carcasses. Both treatment and sex differences may be thought to be large in relation to the standard errors, and analysis shows them to be on the verge of significance; but actual significance cannot be postulated if the 5 per cent level of probability is to be adhered to. If significance in the case of the treatment differences were to be admitted it would be difficult to account for the pigs on treatment B being *shorter* and those on treatment C being *longer* than the pigs on treatment A.

There are significant differences between the litters in respect of length, the pigs in litters 357 and 327 giving, on an average, significantly shorter carcasses than the pigs in the remaining litters.

Table L. *Showing influence of feeding treatments, sex and litter on length of pigs*

(1) <i>Feeding treatment</i>	A		B		C	S.E.
Average length of "middle" (cm.)	78.3		77.6		79.0	±0.41
(2) <i>Sex</i>			Hogs		Gilts	
Average length of "middle" (cm.)			77.8		78.8	±0.33
(3) <i>Litter</i>	357	327	342	208	437	
Average length of "middle" (cm.)	77.3	76.6	79.2	79.5	78.9	±0.52

*"Hot" carcass percentage in relation to feeding treatment, sex and litter*

The data in Table LI for the individually-fed pigs show that *the different feeding treatments have not had any significant influence on the "hot" or "cold" carcass percentages*. The results of the group-fed pigs are in entire harmony with this finding, the averages for treatments A, B and C being 81.9, 82.0 and 81.5 per cent respectively on the basis of "hot" carcass weight. Neither has sex played any significant part

in determining the magnitude of the carcass percentage. The differences between the various litter averages are also of a minor character.

Table LI. *Showing influence of feeding treatment, sex and litter on "hot" carcass percentage*

(1) <i>Feeding treatment</i>	A	B	C	
Average "hot" carcass percentage	81.8 (79.8)*	82.2 (80.2)	81.7 (79.7)	
(2) <i>Sex</i>		Gilts	Hogs	
Average "hot" carcass percentage		82.0	81.8	
(3) <i>Litter</i>	357	327	342	208
Average "hot" carcass percentage	82.8	82.4	81.9	81.3
				81.1

\* Figures in brackets are carcass percentages on basis of factory "dead weight".

*Weight of flare in relation to feeding treatment, sex and litter*

The data for the flares in Table LII (individually-fed pigs) are the averages of the weights of the flares from the two sides. The results demonstrate that *both feeding treatment and sex have been without significant effect on flare weight*. Although the differences between the litter averages are not significant, there is a suggestion of a rough parallelism between these figures and the litter averages for the percentage of fat in the complete rasher. This might be interpreted as meaning that the weight of flare fat may constitute in some degree an index of carcass fatness. This interpretation must be accepted with reserve, however, since litter 208 clearly occupies an anomalous position in the table. A detailed analysis of the results reveals other anomalies in this connexion. It should also be pointed out that considerable practical difficulty attaches to the accurate determination of the weights of the flares and that the flares from the two sides usually differ considerably in weight. For example, in the case of the six pigs in litter 357, the individual flares from the left side showed a range of variation from 670 to 1130 g. (mean=916 g.) whilst those from the right side varied from 592 to 1035 g. (mean=810 g.).

Table LII. *Showing influence of feeding treatment, sex and litter on weight of flare*

(1) <i>Feeding treatment</i>	A	B	C	S.E.
Average weight of flare (g.)	864	840	778	±45.1
(2) <i>Sex</i>		Gilts	Hogs	
Average weight of flare (g.)		778	876	±36.8
(3) <i>Litter</i>	357	327	342	208
Average weight of flare (g.)	863	793	889	735
				856
Average % fat in complete rasher	67.3	63.6	69.7	64.5
				63.7
				± 1.7

*Weight of kidneys in relation to feeding treatment, sex and litter*

The possibility of kidney injury in the case of the pigs subsisting on treatment C, with its abnormally high percentage of protein-rich food, was realized at the time of the planning of the trial. It was decided, therefore, to weigh the kidneys after slaughter of the pigs. The figures for the individually-fed animals in Table LIII represent the average weight of the kidneys from the two sides. It should be pointed out that the left and right kidneys were rarely found to be equal in weight. The discrepancy was not usually marked, but in isolated cases it became very noteworthy. The most discordant case was that of H 951 on treatment C, when the respective weights of the right and left kidneys were 159 and 122 g.

The treatment averages demonstrate that *the high-protein diet C has led to a significant increase in the weight of the kidneys*, the only significant treatment effect so far noted in respect of the post-slaughter results. These heavier kidneys appeared to be perfectly normal, however, no visible signs of actual kidney injury being noted. This finding receives further support from the results of the group-fed pigs, the averages in this case for treatments A, B and C being 113.5, 124.9 and 142.7 g. respectively.

It is of interest to note that treatment B, containing a higher protein supply than would usually be furnished in actual practice, led to no significant increase in the weight of the kidneys. It may be concluded, therefore, that there is little or no danger of kidney hypertrophy in pigs subsisting on the usual rations employed in pig-feeding.

The results in Table LIII show further that sex appears to have had no significant effect on kidney weight, and that among the litters constituting the individual-feeding lay-out, litter 342 had a significantly higher average kidney weight than the others.

Table LIII. *Showing<sup>†</sup> influence of feeding treatment, sex and litter on weight of kidneys*

	A		B		C	S.E.
(1) <i>Feeding treatment</i>						
Average weight of one kidney (g.)	128.5		126.5		143.9	±3.32
(2) <i>Sex</i>			Gilts		Hogs	
Average weight of one kidney (g.)			135.8		130.1	±2.71
(3) <i>Litter</i>	357	327	342	208	437	
Average weight of one kidney (g.)	132.9	127.9	151.7	128.2	124.1	±4.30

*Weight and composition of fillets in relation to feeding treatment, sex and litter*

In the manufacture of bacon it is necessary to remove the fillet, and since the latter consists mainly of the psoas muscle, it was felt that determinations of its weight and chemical composition might shed light on the general muscular development of the whole carcass. Indeed, just as the flare may be regarded as a physiological unit of fatty tissue, so the fillet may be taken as a physiological unit of muscular tissue. The weight data in Tables XLVIII and XLIX were obtained after trimming away all visible fat from the fillets. The water content was found by drying samples of the minced muscle at 105° C. in a hot air oven, the results being adjusted for moisture already lost by evaporation during transport of the fillets from the factory to the laboratory.

Table LIV. *Showing influence of feeding treatment, sex and litter on weight and composition of fillet (individually-fed pigs)*

(1) <i>Feeding treatment</i>	A		B		C		S.E.
Mean weight of fillets in g.	553.5		562.7		582.7		± 27.32
Mean percentage water in fillets	76.4		76.3		76.6		± 0.10
	Hogs   Gilts		Hogs   Gilts		Hogs   Gilts		
Mean percentage fat in fillets	1.82	1.96	2.00	1.60	2.16	1.42	± 0.087
Mean iodine values of fillet fat	65.8		65.3		66.1		± 1.20
(2) <i>Sex</i>			Hogs		Gilts		
Mean weight of fillet in g.			548.8		583.8		± 22.30
Mean percentage water in fillets			76.6		76.3		± 0.08
Mean percentage fat in fillets			1.99		1.66		± 0.056
Mean iodine value of fillet fat			65.4		66.0		± 0.98
(3) <i>Litter</i>	357	327	342	208	437		
Mean percentage water in fillets	76.4	76.5	77.0	76.1	76.4		± 0.13

The data in Table LIV show that although the weights rose consistently as the percentage of protein in the diet was increased, yet *the effect of feeding treatment on fillet weight was not statistically significant*. The gilts gave, on an average, a heavier fillet than the hogs, but again the difference was not significant. There was also an absence of significance in the differences between the litter means in respect of fillet weight.

*Feeding treatment was also without significant influence on the percentage of water in the fillet*, the results for the group-fed pigs being also in harmony with this finding (see Table XLIX). There are, however, significant differences between the litter means, which range from 77.0 per cent of water in litter 342 to 76.1 per cent in litter 208. It will

be noted that the fillets from the hogs had, on an average, a very slightly, though significantly, higher water content than those from the gilts, a somewhat unexpected result in view of the fact that the hogs, on an average, gave fillets having a significantly higher fat content. The finding of a higher fat content in the fillets of the hogs, however, is in harmony with the results already discussed in relation to the comparative fatness of the carcasses from the hogs and gilts.

The interaction of sex and treatment is significant (see Table LIV), implying that there is a progressive rise in the percentage of fat in the fillet as the percentage of protein in the diet was increased in the case of the hogs, and a progressive fall in the case of the gilts. This distinction between the hogs and gilts is not shown by the results for the group-fed pigs, although here again the fillets from the hogs had a distinctly higher fat content. Further inquiry will be necessary to explain this curious result.

*The differences between the mean iodine values of the fillet fat under the three feeding treatments were not significant; neither was there any significant sex distinction in this respect, nor any significance in the differences between the litter means for both the percentages of fat in the fillet and the iodine values of the fillet fat.*

*Firmness of back fat and palatability of bacon in relation to feeding treatment, sex and litter*

The results for the individually-fed pigs in Table LV show that the iodine value of the back fat was slightly though significantly higher in the case of the pigs subsisting on the high-protein diets. This would suggest that the high protein supply had occasioned a very slight softening of the back fat. It would be dangerous, however, to assume that this was a simple and direct effect of high-protein feeding, since the finding is not confirmed by the results for the group-fed pigs (see Table XLIX) and the rations were not perfectly designed for a critical test of this question (see Table I). It will be noted that the high protein diets B and C were derived from the normal diet A by replacing specified percentages of barley meal by a mixture of equal parts of meat meal (5 per cent of fat) and ex. soya-bean meal. It might equally well be argued that the treatment effects on the iodine value of the back fat were due as much to the replacement of the barley meal in this manner as to the feeding of high amounts of protein. The question is being tested more rigidly in a further investigation. Even in the present trial, however, the slight raising of the iodine value of the back fat in the

pigs on treatments B and C was not at all serious, the whole rise from A to C only just coming within the domain of significance.

The back fat from the hogs had, on an average, a significantly lower iodine value than that from the gilts, a finding probably to be related to the greater extent of fattening in the hogs. The differences between the litter means in this respect were not significant.

Tests of flavour, following both frying and boiling, were made on the 120 cuts on which the measurements of fat and lean had been carried out. The flavour of both lean and fat was excellent in every case, and no distinctions whatsoever were noted that might have been attributed to differences of feeding treatment.

Table LV. *Showing influence of feeding treatment and sex on the iodine value of the back fat*

(1) <i>Feeding treatment</i>	A	B	C	S.E.
Mean iodine value of back fat	62.49	63.26	63.87	$\pm 0.397$
(2) <i>Sex</i>		Hogs	Gilts	
Mean iodine value of back fat		62.31	64.11	$\pm 0.324$

*Weight of sides and gammon in relation to feeding treatment, sex and litter*

The weights of the trimmed sides (before curing) for the individually-fed pigs are expressed in Table LVI as a percentage of the carcass weight. It is clear that *feeding treatment has been entirely without influence on the weight of the sides expressed in this manner*. The sex distinction, however, though not very large, is still significant, the sides from the gilts forming a somewhat higher percentage of the carcass weight than was the case with the hogs. There were also significant differences between the litter

Table LVI. *Showing influence of feeding treatment, sex and litter on the weight of the sides (expressed as a percentage of the carcass weight) and the gammon*

(1) <i>Feeding treatment</i>	A		B		C		s.e.
Mean weight of side as percentage of carcass weight	76.0		76.2		76.1		±0.25
Mean weight of gammon in oz.	248.8		247.0		241.8		±3.80
(2) <i>Sex</i>			Hogs		Gilts		
Mean weight of sides as percentage of carcass weight			75.7		76.6		±0.20
Mean weight of gammon in oz.			241.9		249.8		±3.10
(3) <i>Litter</i>	357	327	342	208	437		
Mean weight of sides as percentage of carcass weight	77.0	75.9	75.4	76.0	76.3		±0.32

means, litter 357 showing the highest percentage and litter 342 the lowest.

No significant differences arising from feeding treatment, sex or litter were noted in regard to the weight of the gammon, a conclusion to which the results for the group-fed pigs also point.

*Weight changes of sides during immersion in tank*

The trimmed sides were weighed immediately before and after injection with pickle and after immersion in the pickle tank. It is of interest to note that the average loss in weight of the sides in this process was 0.20 per cent in the case of the hogs and 0.33 per cent for the gilts. That feeding treatment had exercised no significant or even consistent effect on such losses is shown by the mean percentage losses, namely, 0.26, 0.36 and 0.11 per cent, for the pigs on treatments A, B and C respectively.

*Variation within the litter—idiosyncrasy*

Whenever any set of post-slaughter data has been assembled appropriately for the purpose of studying the influence of litter, one feature in every case has stood out with special prominence, namely, the very considerable variation among the results for pigs within the same litter. In the examination, for example, of the factors that might have influenced the fatness or leanness of the carcasses as judged on the basis of the thickness of back fat or the measurements of fat and lean in certain typical rashers, it has been clear throughout that idiosyncrasy, which sums up the inherent characteristics and tendencies of the individual, has exerted a much more potent influence than the factors of feeding treatment, sex and litter. This factor of idiosyncrasy, the display of which is responsible for the high degree of apparently inconsistent and inexplicable variation among individuals in the same litter, has in the main determined the fatness or leanness and other characteristics of the carcasses, quite dwarfing any influence that might have been exercised by the feeding treatments, the latter, although widely different in respect of protein content, having been in nearly all respects without statistically-significant effect. This finding is entirely consistent with experience in human nutrition, members of the same family frequently displaying differences in physical development as striking as they are apparently inexplicable.

The wide range of variation consistently found among the results for the pigs under any one feeding treatment is but to be expected in view



of what has been written about the influence of idiosyncrasy. Such variations are, of course, taken account of in the computation of standard error. The close agreement usually found among the treatment means, despite this considerable range of variation among the individuals in the different feeding treatments, and the fact that statistical analysis of the data has been able to bring consistent sex effects to light, may be taken as proofs of the satisfactory design of the present individual-feeding lay-out.

#### *Application of results to human nutrition*

The present trial has brought to light two facts of outstanding interest: (1) the unusually heavy replacement of cereal by protein-rich food in the diets of bacon pigs has had only the slightest effect on the rate of L.W.I. between weaning and slaughter; (2) the diet containing an abnormally high percentage of protein-rich food has given rise to carcasses neither leaner nor fatter than those arising from a diet containing the normal amount of protein-rich food. From the standpoints of both growth and fattening, therefore, the high-protein diet was scarcely to be distinguished from the normal-protein diet. The reservation should be made, however, that the pigs were fed throughout in accordance with standards designed to give a measure of appetite in pigs at different live-weights; there was thus no deliberate under-feeding.

That a diet extremely rich in protein should be just as fattening as one very much less rich is a significant finding in relation to human nutrition, since it is frequently claimed that a "slimming" diet may be designed when carbohydrate-rich food is generously replaced by food rich in protein. The present trial has yielded no evidence at all in support of this view. Under conditions of feeding up to appetite, it would seem to be immaterial from the standpoint of fattening whether the diet contains excessive or normal amounts of protein. The extent of fattening will be determined rather by the idiosyncrasy of the individual than by the "balance" of the food. If an individual has an inherent tendency to fatness, then apparently the substitution of protein for carbohydrate in the diet will not enable the effects of such a tendency to be obviated.

The energy stored up as fat in the adult is substantially the balance between the energy intake in the food and the output of energy in voluntary and involuntary muscular activity. The process of "slimming" demands a condition of negative energy balance. This may be achieved by a reduction of the energy intake, or by an increase in the energy output, or by both simultaneously. In the case of the pig, for example,

it is known that the deliberate restriction of feeding leads to a leaner carcass than is obtained when pigs are fed to the full limit of appetite. On similar grounds it would be expected that, on a common plane of nutrition, pigs allowed freedom of exercise in the open should yield leaner carcasses than sty-fed pigs, though experimental evidence on this point is at present a little conflicting.

#### *Deamination of surplus protein*

The results of the trial point to the deamination of the amino acids arising, in the high-protein diets, from protein fed in excess of the nitrogen requirements for maintenance and for synthesis of body protein. This is suggested by the significantly heavier kidneys from the pigs on treatment C and has been definitely confirmed in recent metabolism trials carried out by the writers with bacon pigs on different planes of protein intake. The results of these metabolism trials will be published shortly.

Whether the nitrogen-free residues of such deamination changes are convertible directly into body fat, or whether it is merely to be assumed that their oxidation releases an isodynamic amount of carbohydrate for purposes of fat production, is a question that will not be dealt with in this paper. It is sufficient to emphasize that in the present experiment the surplus of protein beyond actual nitrogen requirements must have functioned, directly or indirectly, like carbohydrate in the production of body fat.

#### MAIN CONCLUSIONS

No attempt will be made to give a detailed summary of all the findings of the present investigation, the writers contenting themselves with drawing attention to the following main conclusions:

(1) It has been shown that a feeding treatment providing 12 per cent of fish meal from weaning to 90 lb. L.W., 10 per cent of fish meal from 90 to 150 lb. and 5 per cent of a mixture of equal parts of meat meal and ex. soya-bean meal in the final period supplied all the digestible protein required by Large White bacon pigs for the maximum rate of growth permitted by the net energy content of the ration. An increase in the protein supply beyond these levels did not lead to an increased rate of L.W.I., not even in the period from weaning to 90 lb. L.W., when the influence of extra protein in improving the rate of growth might have been expected to be most clearly manifested. Indeed, an increase in the percentage of protein-rich food to an abnormally high level

(32 per cent in the weaners' ration falling to 25 per cent in the finishing ration) caused a small though significant depression in the rate of L.W.I. and a slight increase in the amount of meal required per lb. L.W.I., this being particularly noticeable in the period from 90 to 150 lb. L.W.

(2) Statistical analysis of the results of the individual-feeding trial demonstrated the significance of the slight drop in rate of L.W.I. in passing from the normal diet to one abnormally rich in protein. The individual-feeding lay-out adopted in the present trial enabled the comparison of the three feeding treatments to be carried out with an accuracy comparable with that associated with modern replicated experiments on agricultural crops.

(3) The group-feeding trial, carried out side by side with the individual-feeding trial, gave results affording general support to the findings of the individual-feeding experiment. Owing to the relative insensitivity of the group-feeding lay-out, however, it was not possible to show that the effects of the different feeding treatments were statistically significant. Despite this lack of sensitiveness, it is suggested that the ideal experimental lay-out is one that submits a given problem to investigation by the group-feeding technique side by side with the individual-feeding method. The group-feeding trial is the link between practical feeding and scientific experimentation and is to be regarded as a verification under practical conditions of the results of the individual-feeding trial. By itself, when the expected differences to be investigated are small, the group-feeding technique may be of very limited value. When carried out side by side with the individual-feeding trial, however, it may, as in the present experiment, constitute an important confirmation.

(4) In comparing the thriftiness of the pigs under the conditions of group feeding and individual feeding, it was found that, over a L.W. increment of 151 lb., the individual feeding led to an average saving of 40 lb. of meal per head, i.e. 7.2 per cent of the average meal consumption per head of the group-fed animals. The significance of this finding in relation to the problems of pig-feeding management is discussed fully in the text.

(5) The results of the individual-feeding trial furnished very suitable material for investigating the influence of sex on L.W.I., since in each of the five pens were six pigs from a given litter, a gilt and a brother hog being kept on each of the feeding treatments A, B and C. Statistical analysis of the results demonstrated a slight yet significant difference in the rate of L.W.I. in favour of the gilts. A further significant sex difference is the finding that, over the whole period of feeding, the gilts

required on an average a smaller amount of meal per lb. L.W.I. than the hogs.

(6) The extremely wide variations in feeding treatment, involving a far-reaching replacement of cereal by protein-rich food, were without significant effect on the thickness of back fat and belly streak. Neither did the feeding of the high-protein rations lead to the production of leaner carcasses, as judged on the basis of back fat and of the percentages of fat and lean in three typical rashers from the belly, back and gammon. The character of the feeding was also without significant influence on (a) the size of the "eye" muscle and the extent of fat infiltration into the area within the line of connective tissue; (b) the length of the pigs as measured from the front rib to the *pubis symphysis*; (c) the carcass percentages; (d) the weight of the flares.

(7) Treatment C, containing the highest percentage of protein-rich food, led to a significant increase in the weight of the kidneys. The latter, however, had a perfectly normal and healthy appearance. Treatment B, which lay, in respect of protein, between treatment A (normal protein supply) and treatment C, did not lead to such increase in kidney weight, and it is concluded that there is no danger of kidney hypertrophy in pigs subsisting on the usual rations employed in pig-feeding.

(8) Investigation of the weight and composition of the fillet (psoas muscle), which represents a physiological unit of muscular tissue, showed that the high-protein feeding had been without statistically-significant influence on the weight of the fillet or on the percentage of water in this tissue. Neither was there any significant difference between the mean iodine values of the fillet fat, nor between the mean weights of the sides (expressed as a percentage of the carcass weight) and the gammons under the three feeding treatments.

(9) The results for the iodine values suggest that the high-protein feeding may have led to a very slight softening of the back fat. The effect, however, was too small to be of practical significance, and reasons are given to show that it may have been due to factors other than the increased protein supply.

(10) The flavour of the bacon was excellent in every case. No distinctions whatsoever were noted that might have been attributed to differences in feeding treatment.

(11) The findings in relation to the influence of sex on carcass quality and conformation were of a more positive character than those relating to the effect of feeding treatment. Statistical analysis of the post-slaughter results showed that the gilts produced somewhat leaner

carcasses as a whole than the hogs. Although the gilts had a tendency to produce a slightly longer carcass and a somewhat heavier fillet, the differences between them and the hogs in these respects were not significant. The fillets of the hogs had, on an average, a slightly higher moisture content than those from the gilts, a somewhat unexpected finding in view of the fact that the hogs gave fillets having, on an average, a significantly higher fat content. Although there was no significant distinction between the mean iodine values of the fillet fat from the hogs and gilts, the back fat of the hogs had, on an average, a lower iodine value than that of the gilts. The sides from the gilts formed a slightly, though significantly higher percentage of the carcass weight than was the case with the hogs, but no such distinction was found in respect of the weights of the gammons.

(12) Sections of the paper are devoted to (1) the influence of litter and of idiosyncrasy; (2) deamination and utilization in fattening of surplus protein; (3) application of results to the problems of human nutrition.

(13) Further sections deal with the significance of the results in relation to various grading problems, such as (1) thickness of shoulder fat as a criterion of fatness along the whole length of the back and of general fatness of carcass; (2) the factory measurement of belly streak as an index of general belly thickness; (3) weaning weight and rate of L.W.I. in relation to grading results and to size of "eye" muscle; (4) grading measurements in relation to length of pig; (5) leanness of carcass in relation to its length.

(14) It should finally be emphasized that although the findings of this investigation are probably applicable to pig-feeding in its widest sense, they are, strictly speaking, referable only to the particular strain of Large Whites forming the experimental animals in the present trial, and possibly only to animals of this strain in so far as they subsist on rations made up from the foods used in the present feeding treatments.

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# THE INFLUENCE OF TEMPERATURE AND AVAILABLE NITROGEN SUPPLY ON THE GROWTH OF PASTURE IN THE SPRING

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(With Nine Text-figures)

## INTRODUCTION

IN recent years a number of workers have investigated the influence of nitrogenous fertilizers on the growth of pastures. In some cases they have claimed that such fertilizers lengthen the grazing season by producing grass earlier than normal in the spring, but no research has been carried out on the factors controlling this spring growth. Watson *et al.* (15) in a study of the influence of nitrogenous fertilizers on the seasons' yield of rotationally grazed pasture at Jealott's Hill, showed that in 1 year ammonium sulphate applied at the rate of 23.2 lb. nitrogen per acre in March enabled the treated plots to be grazed some 14 days earlier than the control. Richardson (13) found that 44.8 lb. nitrogen per acre gave marked increases in yield. Woodman & Underwood (16), however, claimed that ammonium sulphate (14.5 lb. nitrogen per acre) produced no strikingly higher yields.

The present investigations,<sup>1</sup> which were started in 1930, have been carried out on pastures at Jealott's Hill, where the soil is a heavy loam. A study has been made of the influence of nitrogenous fertilizers on (i) the growth of pastures in the spring, (ii) the changes in the nitrogen content of the herbage, and (iii) the variation in the inorganic nitrogen content of the soil.

The experiments were laid out on a number of different fields which varied in their botanical composition. In four of the pastures (Nuptown, Hawthorndale, Beal's Field and Lodge Meadow) the dominant grass was *Lolium perenne* and the subdominants *Poa trivialis* and

<sup>1</sup> Papers summarizing some of the results of this investigation have been read at meetings of the Biochemical Society in June 1932, and the International Soil Science Congress at Oxford in July 1935.

*Agrostis* spp. In another field (Innings) the sward consisted in the main of *Poa trivialis* and *Cynosurus cristatus*, while trials were also conducted on a neglected pasture (Nursery Meadow) consisting of *Festuca ovina* with some *Agrostis* spp.

In studying the influence of nitrogenous fertilizers on the growth of pasture in the spring, two practical aspects of pasture management must be considered. On many farms, especially on heavy land, the physical condition of the soil, particularly in wet springs, may not allow of grazing by stock before a certain date in the season. In other localities, where the land is lighter, stock can be turned out as soon as there is sufficient grass, and therefore earliness is a factor of real practical importance. Under both conditions the problem is the same, since early grass is interrelated with abundant grass. The experiments described in this paper are of two main types; an attempt has been made to measure respectively (i) the *increase in yield* due to nitrogenous manuring, the grass being cut at a time when cattle would normally be turned out, and (ii) the "degree of earliness" produced by nitrogenous manuring, i.e. the *number of days* ahead of the control that the treated pasture reaches the "grazing stage".

#### INFLUENCE OF NITROGEN SUPPLY ON SPRING GROWTH

*Experimental technique.* The procedure adopted for the two series of experiments was somewhat different. In the "increase in yield" experiments, in which the plots were first harvested in early May, the lay-out consisted either of Latin squares or randomized blocks, the yield data obtained thus being capable of statistical treatment according to the methods of Fisher(7). The standard plot size was 0.005 acre, generally square. The nitrogenous fertilizers were applied in late February or early March. The produce from each whole plot was harvested by means of a scythe in 1930-1 and by a special mower in 1932-5. The produce was weighed in the field and a subsample taken per plot for the determination of the percentage dry matter.

In the "degree of earliness" experiments, the growth of the pasture was measured by periodic subsampling of large plots. In 1930, at each subsampling, 15 quadrats each 1 sq. yard in area, were selected at random for each treatment, the herbage cut by means of sheep shears, and the dry weights determined. This method was discarded in 1931 on account both of the labour involved and of the relatively large variation in the individual quadrat yields. More accurate data were obtained by cutting ten randomly selected strips (15 by 1.25 ft.) by means of a lawn



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mower. In 1930-1 there was no replication of the larger plots from which the subsamples were taken, but in 1932, 1934 and 1935 there were eight replicates, and a random strip was cut from each plot at each cut.

### EFFECT OF NITROGENOUS FERTILIZERS ON YIELD

The experiments in this series can be divided into two sections. In one, a comparison was made of the results of applying in all 69.6 lb. of nitrogen per acre, as one, two or three dressings during the season. In the other series the influence of the form of the nitrogen was investigated, a number of different nitrogenous fertilizers being applied, in three dressings, each of 23.2 lb. nitrogen per acre. When only a single dressing was given this was applied in February or early March; for two dressings the times of application were March and May, and for three dressings, March, May and July. These experiments were designed to study the effects of nitrogenous fertilizers on yield of pastures throughout the grazing season, but in this paper only the effects on spring growth are considered. The effects on the season's yields will be dealt with elsewhere.

Since it is customary at Jealott's Hill to turn out the dairy herd in late April or early May, the plots were cut for the first time at this period. The yields obtained in the spring, following upon February or March applications of nitrogen at different rates, are given in Table I. It is seen that the response to nitrogenous manuring varied considerably with the season. An application of 1 cwt. per acre of ammonium sulphate (23.2 lb. N per acre) significantly increased the yield by 41, 20, 74 and 87 per cent. in 1930-3, but in 1934 and 1935 the increases of 23 and 28 per cent. were not significant. Trebling the rate of application to 69.6 lb. nitrogen per acre lead to considerably greater increases, viz. 105, 129, 121, 200, 79 and 74 per cent. in the 6 years. In 1932-5 sodium nitrate gave results similar to those obtained with ammonium sulphate.

Table I. *Yields of herbage in spring after treatment with nitrogenous fertilizers. Cwt. dry matter per acre*

Treatment	Rate of application lb. nitrogen per acre	Nursery Meadow		Innings					Mean 1932-5
		5 May 1930	5 May 1931	17 May 1932	3 May 1933	2 May 1934	17 April 1935		
Control	—	7.16	4.47*	4.44	5.42	7.00	8.44	6.33	
Ammonium sulphate	23.2	10.06	5.35	7.74	10.15	8.60	10.83	9.33	
"	34.8	10.68	—	—	—	—	—	—	
"	46.4	—	7.82	7.51	13.93	11.60	11.66	11.16	
"	69.6	14.09	10.21	9.39	16.28	12.53	14.70	13.23	
Sodium nitrate	69.6	—	—	10.48	14.54	13.46	14.39	13.22	
Significant difference ( <i>P</i> = 0.05)	—	1.20	1.19	2.73	2.75	2.71	3.44	—	

\* The 1931 experiment was a continuation of the 1930 experiment, but the plots receiving in the spring of 1930 34.8 lb. nitrogen per acre, received in 1931 46.4 lb.

When the first experiment was started in 1930 it was intended to carry it on over a number of years on the same site. By the middle of 1931 it was, however, realized that constant cutting had produced a marked deterioration of the pasture varying in extent with different treatments. In 1932 the whole plan of the experiments was altered. In the 1930-1 experiment it was noticed that the chief defects of cutting a series of plots every time they reached the grazing stage for more than one season, were the formation of a mat and a depression of the clover (*Trifolium repens*) on the plots receiving nitrogenous fertilizers. It was considered that the formation of a mat and, to some extent, the depression of the clover, was due to the absence of treading by stock and other effects of grazing not simulated by cutting. In order to minimize this disturbing factor, in 1932 three areas were reserved for the experiment, which was laid out successively on a different one of the three each year. Thus in every 3 years the plot area was cut only during one, and was grazed during the other 2 years. While it was found that with such a scheme there was little tendency for a mat to form, the differential depression of clover was not entirely eliminated.

The maintenance of an equal clover content on all plots is a problem of no little difficulty. In the first place, cutting with a scythe or hay mower is very different from the selective grazing of stock. In addition, with cutting, none of the plant nutrients in the herbage is returned, as is the case with animal droppings. Finally, it has been shown by Martin Jones(8, 9) and by the author(2) that nitrogenous manuring does not necessarily lead to a suppression of the clover, provided that the pasture is kept close-grazed during the late spring and early summer. In experimental plots, which are cut for yield measurement, if all the plots are cut when the manured plots are at the grazing stage, then the control plots are cut at less than the grazing stage and receive the equivalent of hard grazing, which is favourable to *T. repens*. If, on the other hand, all cutting is delayed until the controls reach the grazing stage, then the more advanced stage of growth which, by that time, has occurred on the treated plots, tends to depress the clover. These difficulties could be to some extent overcome if each plot was cut separately when it reached the grazing stage. This method entails two disadvantages, viz. greatly increased labour in cutting and the impossibility of statistically analysing the data for individual cuts.

In the second section of this series of experiments, the response of pasture to a number of different nitrogenous fertilizers was investigated. Apart from one experiment in 1931, the design of all experiments was

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similar, viz. to measure the increase in yield obtained by applying three dressings each of 23.2 lb. nitrogen per acre in February, May and July. The experiments were carried out in Nursery Meadow in 1930-1 and in Innings in 1932-4.

In 1930 there was a single experiment in which ammonium sulphate, nitro-chalk, urea and calcium nitrate were compared, and this experiment was repeated in 1931 on the same site. In that year another experiment was also laid out in which ammonium sulphate and calcium nitrate were used at two separate rates, together with urea at the higher rate. In 1932, as with the experiments already mentioned, three separate areas were reserved for the experiment. In this case, however, the experiment was laid out simultaneously on all three areas, and the appropriate fertilizer treatments were applied each year on all three lay-outs. In each of the 3 years, however, a different one was cut, the other two being grazed by stock without any attempt to record the plot yields. This method of manuring was adopted with the object of accentuating the changes in the soil properties brought about by the various fertilizers, since this aspect was also being studied. Applying nitrogen to the two grazed replicates had, however, certain disadvantages. Since the whole area was grazed by stock, the droppings tended to be equally distributed over the plots. Such a distribution favoured the controls, since the amount of droppings that fell on these plots was not in proportion to the smaller amount eaten from them. Moreover, as the plant nutrient content of the faeces obtained from grazing the nitrogen-treated plots was higher than that of those obtained from untreated areas, there was a further disproportion in the return of nitrogen to the controls, since the majority of the area was dressed with nitrogenous fertilizers. Finally, grazing in small fenced-in areas tended to be selective, while the palatability of the herbage was differentially altered by manuring.

The yields obtained in May following the application in February or early March of 23.2 lb. nitrogen per acre are given in Table II. The results show that of the eight nitrogenous fertilizers tested, no single one has given consistently better results than the others in every year. In 1930 urea gave a significantly poorer yield than ammonium sulphate or calcium nitrate; in 1931 there was no difference between treatments. In 1932 the results were somewhat anomalous; sodium nitrate increased the yield to a significantly greater extent than calcium nitrate, urea, ammonium sulphate or monammonium phosphate, but the yields obtained with diammonium phosphate, ammonium nitrate and nitro-chalk were not significantly different from sodium nitrate. In 1933, when at

the time of cutting the herbage was at a somewhat advanced stage of growth, the yields from the different treatments did not differ significantly from one another. In 1934 calcium nitrate gave a greater yield than nitro-chalk, ammonium nitrate, urea or diammonium phosphate, but was not significantly different in its effect from sodium nitrate, ammonium sulphate or monammonium phosphate.

Table II. *Yields of herbage in spring after treatment with different nitrogenous fertilizers. Cwt. dry matter per acre*

Treatment	Rate of application of nitrogen lb./acre	Nursery Meadow			Innings			Mean yield 1930-4
		5 May 1930	20 May 1931	5 May 1931	17 May 1932	9 May 1933	4 May 1934	
Control	—	7.08	9.96*	4.54	6.24	21.25	13.21	11.55
Ammonium sulphate	23.2	11.86	13.20	7.15	8.38	25.20	17.43	15.21
Monammonium phosphate	23.2	—	—	—	7.09	26.93	17.39	—
Diammonium phosphate	23.2	—	—	—	9.39	26.63	17.02	—
Ammonium nitrate	23.2	—	—	—	8.73	23.45	16.48	—
Nitro-chalk	23.2	10.32	12.80	—	9.57	26.43	16.02	15.03
Urea	23.2	9.75	13.55	—	7.56	25.10	16.61	14.51
Calcium nitrate	23.2	11.21	12.46	—	8.50	26.10	19.01	15.46
Sodium nitrate	23.2	—	—	7.13	10.88	24.96	17.98	—
Ammonium sulphate	69.6	—	—	9.56	—	—	—	—
Urea	69.6	—	—	9.14	—	—	—	—
Sodium nitrate	69.6	—	—	8.34	—	—	—	—
Significant difference ( $P=0.05$ )		1.45	1.58	1.07	2.37	4.53	1.82	—

\* A continuation of the 1930 experiment.

Ammonium sulphate, nitro-chalk, calcium nitrate and urea at 23.2 lb. nitrogen per acre were compared in all 5 years. The absence of any consistent marked difference between them in their effect on the yield of the first spring cut is well shown by the average for the 5 years, given in the last column of Table II, viz. an increase of 2.96-3.91 cwt. of dry matter per acre. From the data in Table I the average effect of ammonium sulphate at the rate of 23.2 lb. nitrogen per acre for the 6 years 1930-5 inclusive, was an increase of 2.63 cwt. of dry matter per acre. For the 4 years 1932-5 (last column Table I), ammonium sulphate at the rate of 23 lb. nitrogen per acre increased the yield by 3.00 cwt. of dry matter per acre. By doubling or trebling the dressing the response was increased by about 60 and 130 per cent. respectively. At neither the lowest nor the highest rate of dressing was there anything to choose between the average effect of either ammonium or nitrate fertilizers.

In spite of these well-defined and consistent results, when the data are averaged over a number of years, and allowing for the special circumstances affecting the comparison of the 1930 and 1931 data, the variation from year to year is a marked feature of the results. It was the object

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of the experiments about to be described to elucidate the factors responsible for this seasonal variability.

### INFLUENCE OF NITROGENOUS MANURING ON RATE OF GROWTH OF PASTURE

In each of the years 1930-5, with the exception of 1933, an attempt was made to measure how many days ahead of the control, the nitrogen-treated pasture reached the "grazing stage". In 1930 a simple unreplicated trial was laid out to determine what effect a dressing of 23.2 lb. nitrogen (as ammonium sulphate) per acre had on the growth rate. In Fig. 1 the data are plotted as growth curves for the spring period of the treated and control pastures, each point representing the mean yield obtained from fifteen 1 yard sq. quadrats. In addition, the daily soil temperature at a depth of 4 in. is shown; the observations were made at 9 a.m. It was realized that a single observation was by no means ideal for correlating growth with temperature changes. The "4-in." soil temperature has, however, certain advantages; the daily fluctuations are less marked than for the maximum or minimum air temperature or the grass minimum, while for investigating any relationship between nitrogenous manuring and the soil micro-organisms it is the most suitable.<sup>1</sup> In some years besides noting the soil temperature at 9 a.m. other observations were made at 1 and 5 p.m. It was found that the 5 p.m. reading was almost invariably the highest; the mean rise from 9 a.m. to 5 p.m. was 5.1° F. in 1934 and 4.6° F. in 1935. The fluctuations in the daily 1 and 5 p.m. observations were greater than the figures obtained at 9 a.m.

From Fig. 1 it is seen that there had been no response to nitrogen, and presumably no appreciable growth, up to the end of March, low temperature probably being the controlling factor; only once did the soil temperature exceed 45° F., the average for the 15 days being 40° F. During April the soil temperature ranged between 40.5 and 53° F., being within the limits of 42-47° F. on all but 12 days, with an average for the month of 46° F. The nitrogen-treated pasture under these conditions grew at a faster rate than the control. In May, when the soil temperature was above 50° F. for most of the time, the average for the first 14 days being 51° F., the growth rates would appear to have become about the same.

From the data given in Fig. 1 it is possible to estimate the "degree of earliness" obtained by applying 23.2 lb. of nitrogen per acre in

<sup>1</sup> For simplicity of reference the words "soil temperature" in the remainder of this paper signify soil temperature at 4 in. depth at 9 a.m. unless otherwise stated.

February. For purposes of comparison, a production of 7.5 cwt. of dry matter per acre has been taken as equivalent to the "grazing stage". This figure has been chosen as being intermediate between the grazing stage for sheep (5.0 cwt. per acre) and the grazing stage for cattle (10 cwt.). On this basis, the earliness obtained by nitrogenous manuring amounted to some 13 days, as shown by the length of the line at the yield level of 7.5 cwt. per acre, which is intercepted between the two graphs.

In 1931 a similar experiment to that of 1930 was conducted on the same site, but with the position of the control and treated plots trans-

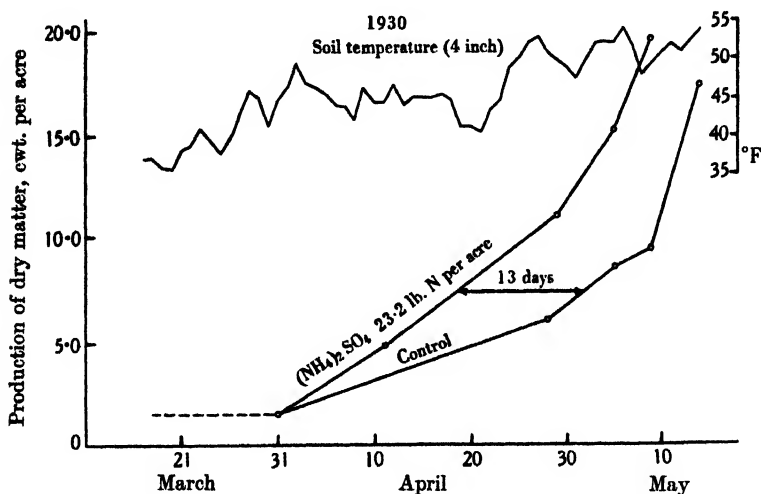


Fig. 1. The influence of soil temperature and nitrogenous manuring on the growth of pasture in the spring.

posed. In addition, however, to comparing untreated pasture with that receiving 1 cwt. of ammonium sulphate per acre, a further treatment was included, viz. 3 cwt. of ammonium sulphate per acre. The growth curves plotted from data obtained from random mower cuts (see p. 621) together with the soil temperatures are shown in Fig. 2. Again, there was no appreciable growth during March when the soil temperature exceeded 45° F. on only 3 days, and the average for the 19 days concerned was 41° F. During April the soil temperature ranged between 39 and 48° F., being outside the limits of 42 and 47° F. on 13 days, with an average of 45° F. for the month. Until the end of the month the growth rate on the manured plots was greater than on the control. Growth was, however, so slow that on none of the plots was the yield

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of herbage high enough to give an "early bite" of 7.5 cwt. dry matter per acre until the beginning of May, when the temperature rose so sharply that there was a very rapid increase in the growth rate on both the treated and untreated areas. As a result of these conditions the "degree of earliness" obtained was small; about 5 days for an application of 69.6 lb. of nitrogen per acre and negligible for 23.2 lb. For an "early bite" for sheep, of 5 cwt. dry matter per acre, however, the more heavily manured plots were ready for grazing 19 days sooner than the control.

As previously mentioned the results for 1930 and 1931 were obtained by subsampling single large plots. It might be argued that the observed differences could be ascribed to variation, both in soil fertility and plant population. This objection was realized before the 1930 experiment was started, but as both the error of the sampling technique and the amount of labour involved were unknown, it was decided to attempt, at first, a simple trial. Observations suggested that the apparent nitrogen effect was a real one, since there was a marked and uniform difference in growth between the contiguous treated and untreated areas. In 1931, when the experiment was carried out on the same site with the position of the treated and untreated plots reversed, the poor response to ammonium sulphate might be explained on the grounds that the pasture on the original treated area in 1930, i.e. the control in 1931, was superior. On the other hand, the poor results in 1931 were more probably due to the fact that the experiment was carried out for 2 years on the same site. Investigations then in progress at this Station have subsequently shown that hard grazing in the winter and spring in consecutive years is not advisable, being likely to weaken the grasses so that they are less responsive to manuring in spring (Martin Jones(8, 9), Blackman(2)). In 1932, 1934 and 1935 the areas designed for similar experiments were rested during the autumn and winter; in addition each experiment was in eightfold replication.

In 1932 the investigation was confined to studying the effect of 3 cwt. of ammonium sulphate per acre applied in early March. The growth curves obtained are shown in Fig. 3. During March the soil temperature fluctuated between 33 and 46° F., being below 45° F. until the last 2 days of the month, with an average for the last 21 days of 38.5° F. and no growth took place. During April the soil temperature ranged between 40 and 51° F., being outside the limits of 42–47° F. on only 7 days with an average of 44.2° F. for the month. Under these conditions once more the treated pasture grew faster than the control. At the end of the month the soil temperature rose rapidly to over 50° F.,

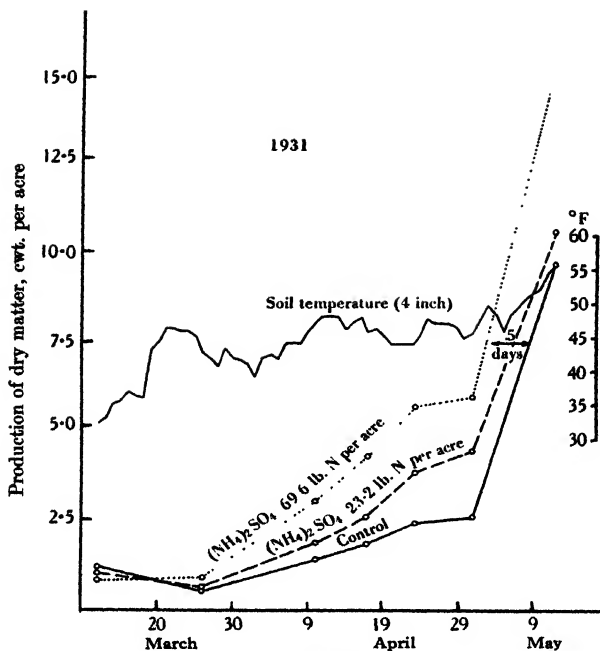


Fig. 2.

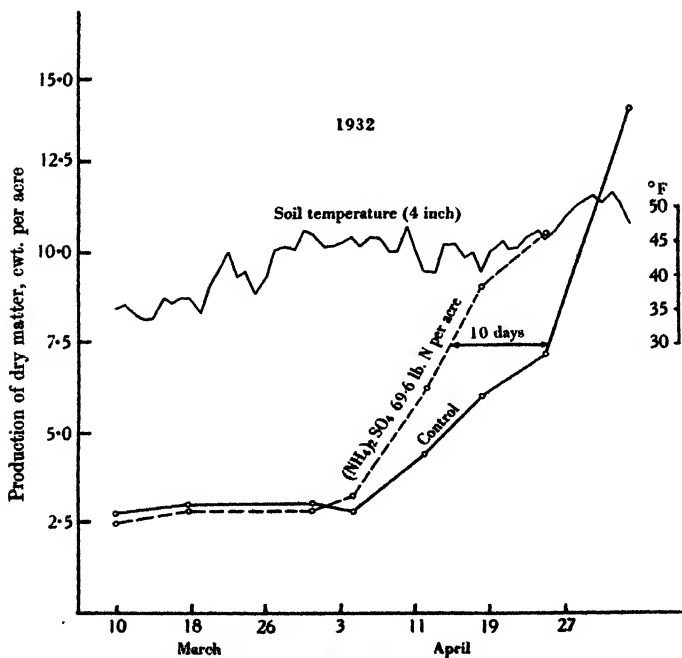


Fig. 3.

Figs. 2-3. The influence of soil temperature and nitrogenous manuring on the growth of pasture in the spring.



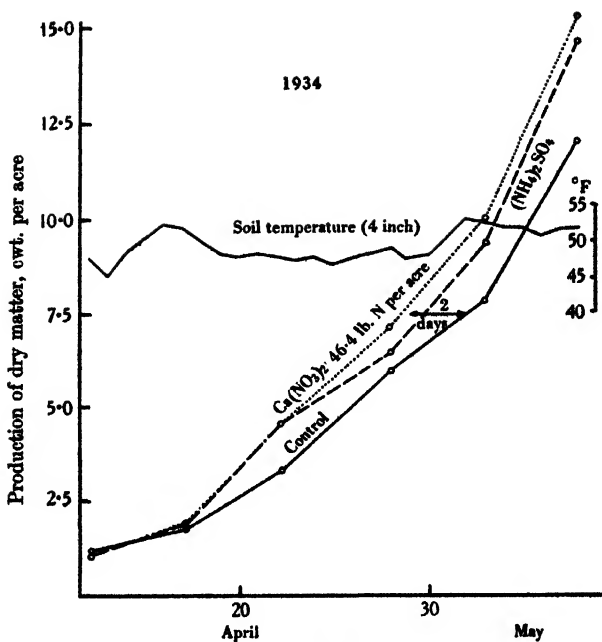


Fig. 4.

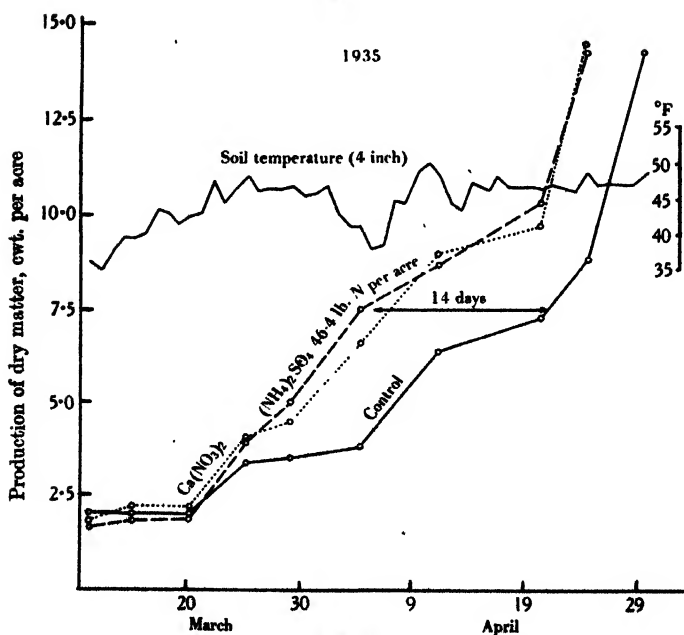


Fig. 5.

Figs. 4-5. The influence of soil temperature and nitrogenous manuring on the growth of pasture in the spring.

and at the same time the growth rate on the control increased sharply. Under these conditions the treated pasture reached the grazing stage (7.5 cwt. dry matter per acre) some 10 days ahead of the control.

In the 1934 experiment in addition to ammonium sulphate, calcium nitrate was also included. The replication, as in 1932, was eightfold, but the quantity of nitrogen applied was reduced to 46.4 lb. per acre. The experiment was not started until early in April. It is seen from the low value of the initial cut (*vide* Fig. 4) that little growth can have taken place earlier in the spring when the soil temperature varied between 33.8 and 43.8° F. from 1 March to 10 April. Between 10 and 14 April the soil temperature rose rapidly from 40.8 to 48.5° F.; from that time until the end of April it varied between 47 and 52° F., never falling below the lower figure and with an average temperature for the period 10–30 April of 48.6° F. Under these conditions the herbage on the control grew nearly as rapidly as that receiving either ammonium sulphate or calcium nitrate, and in consequence the “degree of earliness” was only a matter of about 2 days.

In 1935 a similar experiment to that of 1934 was laid down, the only difference being that the nitrogenous fertilizers were applied 1 month earlier, i.e. 11 March. The growth curves obtained are shown in Fig. 5. As in previous years no growth took place when the mean soil temperature did not exceed 42° F. After 20 March, with a rise in temperature above this level, growth commenced on both the treated and control areas. From 20 March to 15 April the soil temperature was below 47° F. on all but 6 days, with an average value of 45° F. During this period the manured herbage grew at a greater rate than the control. After 15 April the soil temperature never fell below 46° F. and the average for the 14 days from then until the end of the experiment was 47.1° F. During this period the growth rate of the herbage on the control showed a sharp increase to about the same final rate as on the manured plots. Between 24 and 29 April, as in previous years, there occurred a “flush” of growth when the soil temperature varied between 47 and 49° F.

These results are discussed in more detail later in this paper. At this point it suffices to call attention to the fact that large differences were observed, in different seasons, in the “degree of earliness” obtained; the longer the soil temperature remained between 42 and 47° F. the greater was the advantage of the manured plots over the control. Differences in the sward and in the rate of manuring must, however, also be taken into account in considering these results.

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Another point of particular interest in relation to the data presented in the next section is the similarity in the rate of action of ammonium sulphate and calcium nitrate; the ammonium salt accelerated the growth rate as soon and as much as the nitrate.

### INFLUENCE OF NITROGENOUS MANURING ON THE NITROGEN CONTENT OF THE HERBAGE

In all the experiments so far cited, the nitrogen content of the herbage was determined at each cut. It is not proposed to deal in detail with all the data. In those experiments in which the pasture was first harvested in early May (*vide* Tables I and II), nitrogenous manuring at the rate of 23.2 lb. nitrogen per acre increased the percentage nitrogen content of the dry matter in general by a small amount. With the higher rate of 69.6 lb. nitrogen per acre the nitrogen content was appreciably greater in the dry matter of the manured herbage.

In the "degree of earliness" experiments for which the growth data have already been given, the nitrogen content of each replicate at each cut was determined separately in the years 1932, 1934 and 1935; the data for these years are thus capable of statistical analysis. The changes in total nitrogen content of the dry matter, following upon nitrogenous manuring in the 4 years 1931, 1932, 1934 and 1935, are shown in Fig. 6. In each year the changes were very similar. There was an immediate and significant ( $P < 0.05$ ) rise in the nitrogen content following upon the application of the nitrogenous fertilizer in 1932-5. The uptake of nitrogen by the roots and its subsequent translocation to the shoot can therefore take place at temperatures which inhibit growth, since it has already been shown that in 1932 active growth did not commence until early April (*vide* Fig. 3), while in 1935 low temperature was the factor controlling growth until 20 March (*vide* Fig. 5).

The maximum nitrogen content occurred a few days after growth commenced, i.e. early April in 1931-4 and late March in 1935. The maximum value may be of a high order, e.g. over 5 per cent. when 69.6 lb. of nitrogen was applied per acre, and approximately 4 per cent. when the rate of application was 46.6 lb. per acre. As the stage of growth became more advanced, the difference in nitrogen content between manured and unmanured herbage, as would be expected, decreased.

The marked increase in nitrogen content of the herbage within 4-8 days of the application of ammonium sulphate, both in 1932 and 1935, suggests that much of the added nitrogen must have been absorbed as ammonium ions. In 1935 and more particularly 1932, low soil tem-

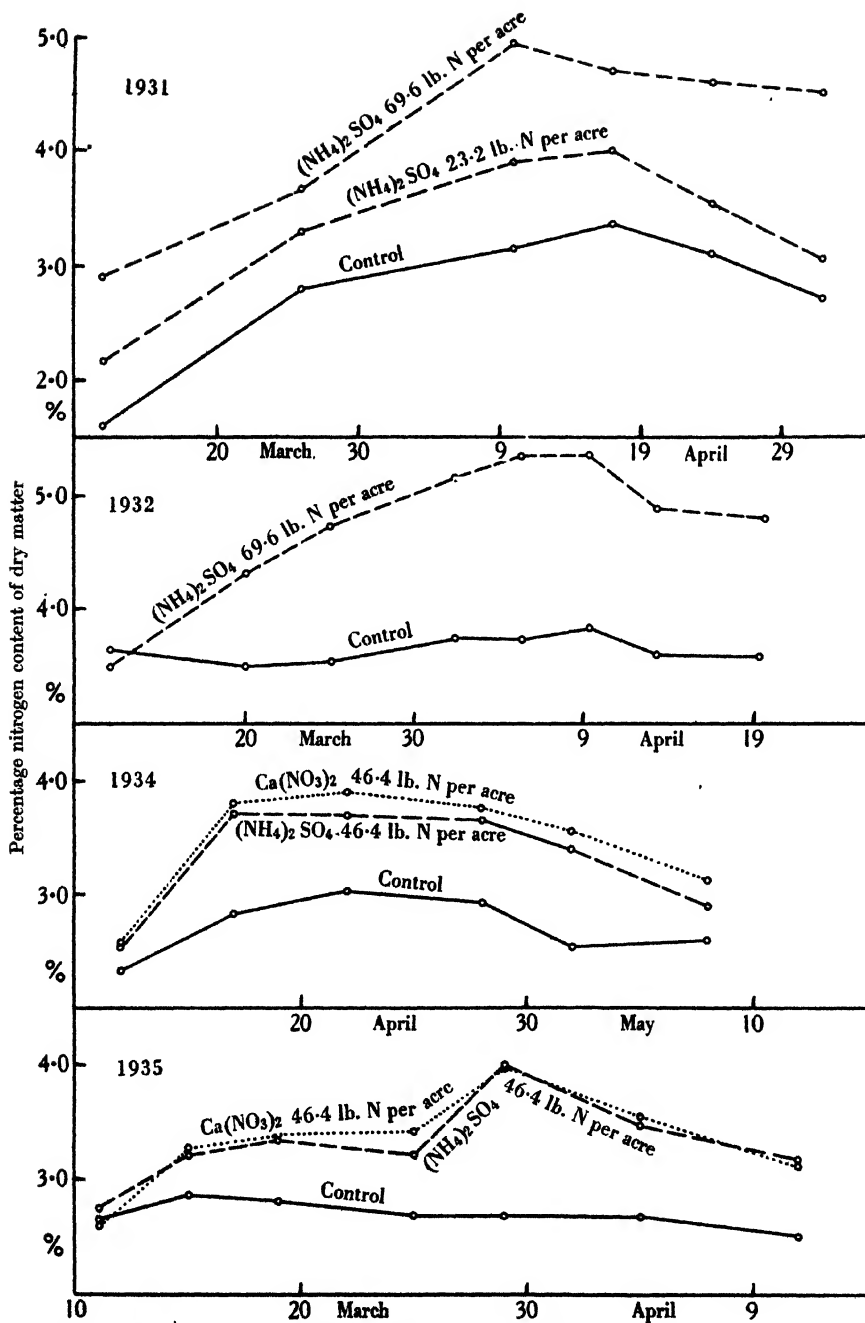


Fig. 6. Changes in the nitrogen content of the herbage following upon manuring with nitrogenous fertilizers in the early spring. The fertilizers were applied on 25 February 1931, 10 March 1932, 12 April 1934, and 11 March 1935.

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perature may be expected to have prevented rapid nitrification. Unless, therefore, active absorption of ammonium ions took place, it would not be expected that in 1935 the increase in the nitrogen content 4 days after application would be the same for ammonium sulphate as that for calcium nitrate; but this was in fact the case. Moreover, as already pointed out, there was no difference between the effects of ammonium sulphate and calcium nitrate on the rate of growth. The increases in nitrogen content observed were not due to any fertilizer still adhering to the foliage, for, in each year, the fertilizers were applied as a dilute solution, while as a further precaution in 1934 and 1935 the plots were subsequently sprayed with water from a hose. Furthermore, in 1935, samples of the herbage taken immediately before and after the fertilizer applications showed on analysis no difference in nitrogen content.

Recent water and pot culture investigations(3, 11) have shown that in plants with a weakly acid sap such as the grasses, ammonium ions on absorption do not accumulate as ammonium salts but react with the carbohydrates to form initially amide compounds and to a lesser extent amino acids. On the other hand, work by Eggleton(6) and other unpublished work at this Station has shown that in grasses nitrate nitrogen on absorption can accumulate as such to a very considerable extent in the herbage. Similar accumulations of nitrate nitrogen in barley are reported by Richards & Templeman(12). It follows, therefore, that by studying in detail the changes in the amounts of the various kinds of nitrogen compounds in the herbage it is possible to ascertain whether any increase in nitrogen content is due to the absorption of ammonium or of nitrate ions. With this object in view such analyses have been carried out on the herbage from the 1932 and 1935 experiments, using the methods of micro-analysis employed by Richards & Templeman(12).

In order that there should be as little change as possible after cutting, the samples after collection were immediately placed in a special steam-heated oven in which the material dried completely within an hour. The analyses were carried out on true composite samples made up of "weighted" proportions from the eight replicates.

The results of the analyses carried out on the dried material from the 1932 "degree of earliness" experiment (*vide* p. 628) are given in Table III. It is seen that 8 days after the application of ammonium sulphate there has been, relative to the control, not only a marked increase in the total nitrogen content, but also a change in the proportions of the different non-protein fractions. After a further 6 days (23 March) these differences were much smaller. In subsequent cuts, although the manured herbage

contained more nitrogen than the control, the proportions of the constituents were similar.

It is seen that manuring with ammonium sulphate led, at the end of 8 days, to only a slight increase in the protein nitrogen. There was, however, a marked rise in the non-protein nitrogen, especially for amide, amino and ammonia nitrogen. After 13 days the protein nitrogen increased at the expense of the non-protein nitrogen.

Table III. *Nitrogen content and nitrogen partition of herbage treated with ammonium sulphate*

Treatment	Date of sampling	Total nitrogen content % of dry matter	Nitrogen fractions as % of total N						Residual non-protein
			Protein	Non-protein	Amino	Amide	NH <sub>4</sub>	NO <sub>3</sub>	
Control	10. iii. 32*	3.66	84.15	15.85	3.58	2.02	0.49	0.79	8.97
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		3.50	83.82	16.18	3.45	3.85	0.37	1.23	7.28
Control	18. iii. 32	3.50	80.93	19.07	3.27	3.38	1.16	0.86	10.4
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		4.30	69.40	30.60	6.72	11.10	4.66	1.52	6.60
Control	23. iii. 32	3.53	83.17	16.83	3.50	1.96	0.34	0.43	10.60
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		4.72	80.18	19.84	2.52	6.30	0.53	2.31	8.18
Control	31. iii. 32	3.76	83.19	16.81	2.16	2.45	0.37	0.43	11.4
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		5.14	79.95	20.05	2.80	5.04	0.27	1.34	10.60
Control	4. iv. 32	3.73	85.19	14.81	2.04	2.92	0.30	0.43	9.12
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		5.33	80.15	19.85	2.30	4.63	0.21	2.51	10.20
Control	8. iv. 32	3.83	81.20	18.80	2.01	2.97	0.16	0.26	13.40
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		5.35	80.10	19.90	2.08	4.65	0.15	1.42	11.60

\* The ammonium sulphate was applied on this date immediately after the samples of herbage were collected for analysis.

Table IV. *Nitrogen content and nitrogen partition of herbage treated with ammonium sulphate and with calcium nitrate*

Treatment	Date of sampling	Total nitrogen content % of dry matter	Nitrogen fractions as % of total N						Residual non-protein
			Protein	Non-protein	Amino	Amide	NH <sub>4</sub>	NO <sub>3</sub>	
Control	11. iii. 35*	2.66	83.26	16.74	2.97	4.14	0.38	0.15	9.10
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		2.77	85.64	14.36	2.56	3.72	0.33	0.15	7.60
Ca(NO <sub>3</sub> ) <sub>2</sub>		2.65	84.14	15.86	2.34	3.81	0.23	0.08	9.40
Control	15. iii. 35	2.89	81.90	18.10	2.10	4.45	0.20	1.00	10.35
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		3.22	72.05	27.95	6.20	8.69	2.73	1.28	9.05
Ca(NO <sub>3</sub> ) <sub>2</sub>		3.28	71.88	28.12	3.33	4.57	0.46	9.47	10.29
Control	19. iii. 35	2.81	84.85	15.15	2.33	3.19	0.11	0.82	8.70
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		3.35	79.58	20.42	3.38	5.79	0.66	2.25	8.34
Ca(NO <sub>3</sub> ) <sub>2</sub>		3.38	80.88	19.12	2.72	3.87	0.18	2.68	9.67
Control	25. iii. 35	2.69	82.57	17.43	2.92	3.34	0.23	0.84	10.10
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		3.22	83.13	16.87	3.95	4.91	0.09	0.81	7.10
Ca(NO <sub>3</sub> ) <sub>2</sub>		3.41	82.26	17.74	3.52	3.46	0.09	2.14	8.53

\* Both the ammonium sulphate and the calcium nitrate were applied on this day, immediately after the samples of herbage were collected for analysis.

The analytical data for the 1935 degree of earliness experiment (*vide* p. 631) are set out in Table IV. It is seen that on 11 March, prior to the

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application of the fertilizers, there was little difference either in the nitrogen content or the constituent nitrogen fractions. Four days after the application of the fertilizers, the nitrogen content of the herbage manured with either ammonium sulphate or calcium nitrate had increased by the same amount. As a result of these increases, the proportions of the various nitrogen fractions were altered, but 14 days after the fertilizer applications the proportions were again, as initially, of the same order. The actual nitrogen content of the control was, however, considerably smaller.

Four days after the fertilizer applications, manuring with either ammonium sulphate or calcium nitrate led to an increase in the non-protein nitrogen, but no increase in the protein nitrogen. Although these increases in the non-protein nitrogen were of the same order for both fertilizers, the increases in the various nitrogen fractions of the non-protein nitrogen were very different. Manuring with ammonium sulphate brought about a marked increase in ammonia and amino plus amide nitrogen, but no appreciable rise in the nitrate nitrogen. Treatment with calcium nitrate, on the other hand, resulted only in a large increase in the nitrate nitrogen and a small rise in the amino plus amide nitrogen. At the end of 8 days there was for both treatments a fall in the non-protein nitrogen and a rise in the protein nitrogen. In the case of the herbage treated with calcium nitrate, the fall in the non-protein nitrogen was due to synthesis into protein of a large proportion of the nitrate nitrogen. In the herbage manured with ammonium sulphate, the decrease in the non-protein nitrogen was brought about by the elaboration of the ammonia nitrogen and part of the amide and amino fractions.

These results offer convincing proof of the direct absorption of ammonium ions from the soil. If there had been no absorption of ammonium ions, as such, but only of nitrate ions following nitrification, then in 1935 the increase in nitrogen content observed shortly after the application of ammonium sulphate should have led to an accumulation of nitrate nitrogen similar to that found for calcium nitrate. The fact that with the ammonium sulphate there was no appreciable rise in the nitrate-nitrogen fraction in the initial period, is considered to be conclusive evidence that most of the nitrogen absorbed was in the form of ammonium ions. The increase in ammonia, amino, and amide nitrogen, obtained in both 1932 and 1935, is further evidence that there was direct absorption of ammonium ions, since it would be expected that this absorption would lead not only to a higher ammonia content, but also

to a higher amide- and amino-nitrogen content due to reaction of the ammonium ions with the carbohydrates.

Eggleton<sup>(6)</sup> has also recently investigated the changes in the nitrogen content of the herbage following upon the application of ammonium sulphate to pasture in May. He observed that within 2 hours there was an increase in the nitrogen content of the herbage, and a higher proportion of ammonia nitrogen than on the control. Moreover, no appreciable rise in the nitrate-nitrogen content took place in the herbage until 9 days after the fertilizer application. The proportions of ammonia and nitrate nitrogen found were considerably smaller than in the present investigation. This difference would be expected, since in May protein synthesis would be more active than in March.

#### THE AVAILABLE NITROGEN CONTENT OF THE SOIL

When these investigations were started in 1930, a search of the literature revealed no previous detailed study of the changes in the ammonia- and nitrate-nitrogen content of grassland soils. It was therefore decided to investigate the changes in the inorganic nitrogen content of the soil following upon the application of nitrogenous fertilizers in the early spring.<sup>1</sup> The first experiment was carried out in 1930, and subsequent experiments were conducted in 1931, 1934 and 1935.

*Experimental technique.* In 1930 a preliminary experiment was carried out on small (9 × 9 ft.) unreplicated plots, and four borings to a depth of 3 in. were taken at each sampling. In 1931 the experiment was in sixfold replication, two borings from each replicate being taken and bulked together for the nitrogen determinations. In 1934 the replication was eightfold, and twenty borings to a depth of 3 in. were taken per plot (250 sq. ft.). The nitrogen determinations were carried out on each replicate. In 1935 the replication was reduced to fourfold, with ten borings per plot (50 sq. ft.), while the nitrogen determinations were again carried out on each replicate. The extraction and determination of ammonia and nitrate nitrogen were made by Olsen's<sup>(10)</sup> methods.

*1930 data.* As this experiment was of only a preliminary nature, the results will not be given in any detail. It was found that the ammonia-nitrogen content of the soil fell very rapidly after the application of ammonium sulphate (23·2 or 69·6 lb. nitrogen per acre) in early March; within a fortnight the levels on the controls and treated areas were of the same order.

<sup>1</sup> Since 1930, papers on this subject have been published by Richardson<sup>(13)</sup>, Eggleton<sup>(5)</sup> and the author<sup>(3)</sup>.



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1931 data. In this trial, laid out in Nursery Meadow, ammonium sulphate and sodium nitrate were each applied at two rates, namely, 23.2 and 69.6 lb. nitrogen per acre. The fluctuations in the soil ammonia- and nitrate-nitrogen contents for the various treatments are shown in Fig. 7.

In the lower half of Fig. 7 it is seen that subsequent to the application of ammonium sulphate, there was a gradual fall in the ammonia-nitrogen

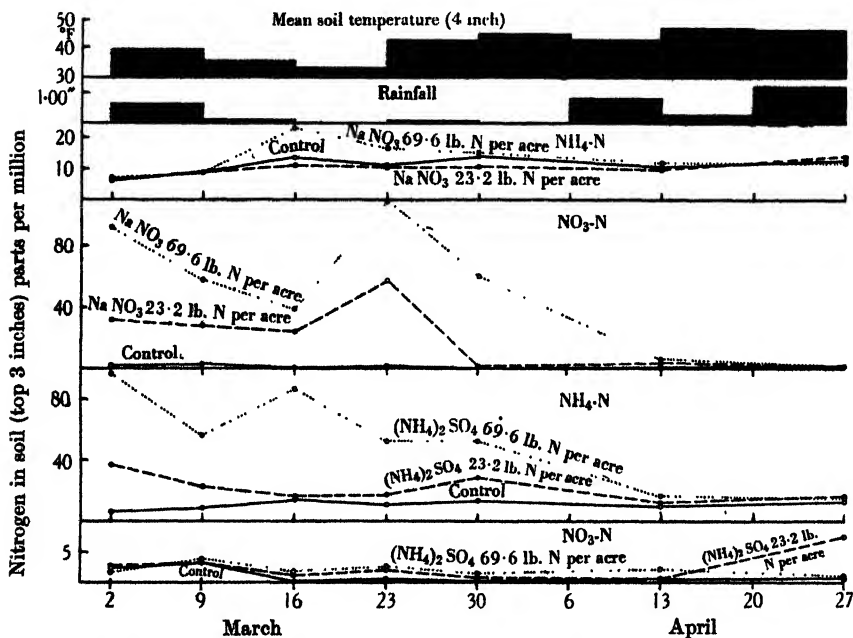


Fig. 7. Changes in the ammonia- and nitrate-nitrogen content of the top 3 in. of soil following upon applications of ammonium sulphate and sodium nitrate. The initial nitrate-nitrogen values for sodium nitrate and the initial ammonia-nitrogen values for ammonium sulphate are shown as black circles. These values were obtained by adding to the ammonia- or nitrate-nitrogen concentrations, determined prior to the fertilizer applications, either 23.2 or 69.6 lb. of nitrogen per acre expressed as parts per million by weight of the soil in the top 3 in. ( $0.75 \times 10^6$  lb.).

content of the soil. With the higher rate of application the ammonia-nitrogen value at the end of the second week was lower than at the end of the third week. By mid-April, however, when the pasture was growing actively, most of the added nitrogen could no longer be found in the soil extract. There would appear to have been little nitrification of the added ammonium sulphate during March. It must, however, be borne in mind that most of the nitrate nitrogen was probably absorbed by the pasture plants.

The changes in inorganic nitrogen content following upon the addition of sodium nitrate at the rate of 23.2 and 69.6 lb. nitrogen per acre are shown in the upper half of Fig. 7. By the end of the second week, with both rates of application, there was a marked fall in nitrate-nitrogen content. In the second week there was an apparent increase in the ammonia-nitrogen concentration on the plots receiving sodium nitrate at the higher rate. In the third week a sharp increase in the level of the nitrate nitrogen occurred with both treatments. In the following 3 weeks with the commencement of active growth, the nitrate-nitrogen concentration was reduced in both cases to the same order as that of the control.

The question arises whether the fluctuations in the 1931 experiment can be attributed to experimental error. It is very unlikely that the changes in nitrate-nitrogen content can be due to losses by leaching and subsequent appearance in the surface layers, since apart from slight rain in the first week rainfall during the next 3 weeks was negligible. Errors due to uneven distribution of the sodium nitrate might be put forward as an explanation, but this is also improbable, since the fertilizer was applied as a dilute solution.

There is considerable evidence that the fluctuations in the nitrate content are real. In the first place, although all the samples were bulked for the chemical analysis, the cores were taken from plots in sixfold replication. The initial ammonia and nitrate contents of the six sets of plots showed a good agreement, the standard error for the ammonia and nitrate values being 3.1 and 11.4 per cent. respectively. Further evidence that the fluctuations for the sodium nitrate applications are real is that the changes in the nitrate-nitrogen level from week to week are in step for both rates of application. It is possible to test statistically whether the agreement in the nitrate changes for the two levels of application is fortuitous: calculations show that the rises in nitrate-nitrogen concentration in the third week are significant ( $P < 0.05$ ).

*1934 data.* In 1934, the soil samples were taken from the experiment in which the "degree of earliness" was measured (see p. 631). The soil ammonia- and nitrate-nitrogen estimations were made on each replicate, while the ammonium sulphate and calcium nitrate were applied at only one rate, namely, 46.4 lb. nitrogen per acre. The results obtained from the analyses of samples taken at 4-day intervals are shown in Fig. 8. It is seen that, as in previous years, there was a marked disappearance of the added nitrogen within a few days of the application, and at the end of 21 days the nitrogen level on the treated plots was the same as on the control.

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In the case of the ammonium sulphate, some nitrification had taken place 4 days after application, since there was a significant rise ( $P < 0.02$ ) in the nitrate-nitrogen concentration between 12 and 16 April, when the mean soil temperature was  $47.8^{\circ}\text{F}$ . The addition of calcium nitrate led, at the end of 8 days, to a significant increase ( $P < 0.01$ ) in the

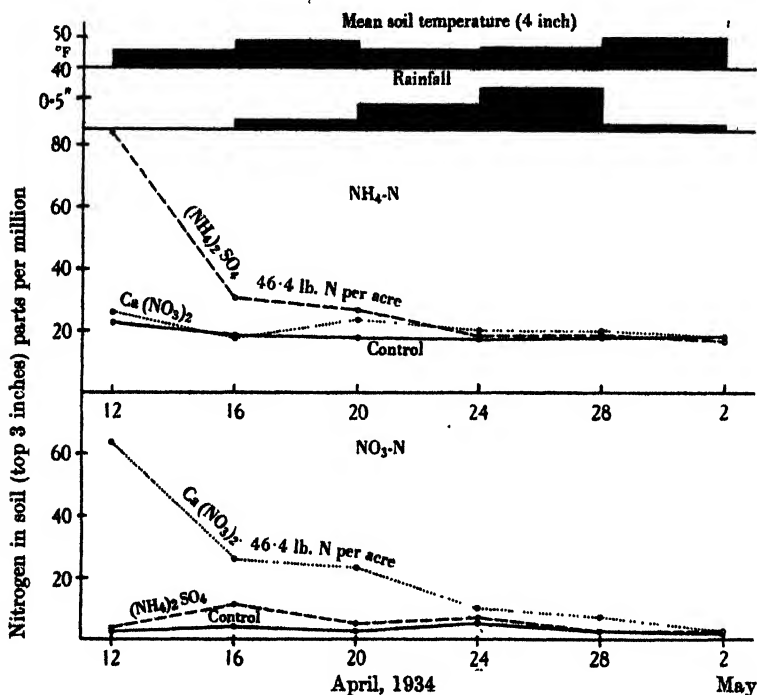


Fig. 8. Changes in the ammonia- and nitrate-nitrogen content of the top 3 in. of soil following upon applications of ammonium sulphate and calcium nitrate. The initial nitrate-nitrogen value for calcium nitrate and the initial ammonia-nitrogen value for ammonium sulphate, shown as black circles, were calculated as in Fig. 7 on a basis of 46.4 lb. of nitrogen per acre.

ammonia-nitrogen concentration. This result is in agreement with the 1931 findings.

**1935 data.** As in 1934, the soil samples were taken from the 1935 "degree of earliness" experiment (see p. 631). In Fig. 9 are shown the changes in ammonia- and nitrate-nitrogen content of the soil following upon the addition of 46.4 lb. nitrogen as ammonium sulphate and calcium nitrate. The graphs show that there has been the characteristic rapid disappearance of both forms of nitrogen in the first 10 days after application.

In the first 10 days, as far as can be judged from the data, there has been little nitrification of the ammonium sulphate, while there has been no rise in the ammonia-nitrogen content on the plots receiving calcium

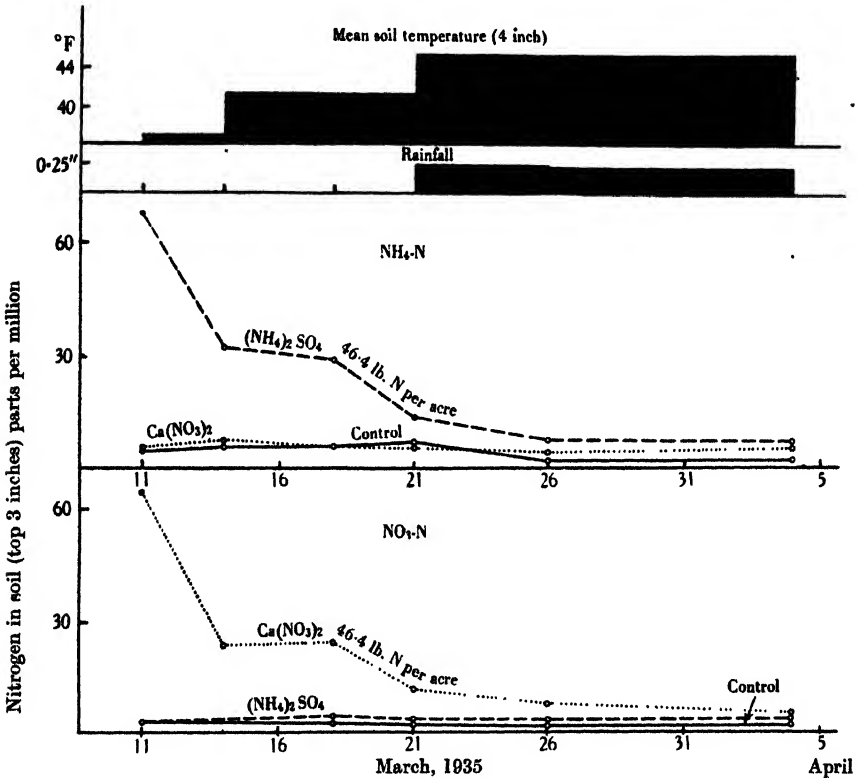


Fig. 9. Changes in the ammonia- and nitrate-nitrogen content of the top 3 in. of soil following upon applications of ammonium sulphate and calcium nitrate. The initial nitrate-nitrogen value for calcium nitrate and the initial ammonia-nitrogen value for ammonium sulphate, shown as black circles, were calculated as in Fig. 7 on a basis of 46.4 lb. of nitrogen per acre.

nitrate. Fifteen days after the application of calcium nitrate, the ammonia-nitrogen concentration was significantly greater ( $P < 0.05$ ) than the control. As, however, the difference was only 2 p.p.m. of nitrogen, the result must be received with considerable reserve, since the method of analysis is only accurate to about 1 p.p.m. of nitrogen.

## DISCUSSION

On the evidence from these investigations it is possible to put forward conclusions as to the principal factors controlling the growth of pasture in the spring. It has been demonstrated that there is a similar trend of changes from year to year. During March, when the soil temperature is low, little, if any, growth takes place. With rising temperature in April, the rate of growth is at first small, but towards the end of the month or early in May there is a rapid increase. The addition of nitrogenous fertilizers to the sward increases the growth rate, particularly during the period just after growth has started.

It seems clear that the changes in growth rate must be associated with the gradual rise in temperature from March to May. In order to interpret more exactly the effect of temperature on the growth of manured and unmanured pasture, the growth rates have been calculated for the successive cuts in each year. For this purpose the logarithmic formula put forward by V. H. Blackman<sup>(4)</sup> has been used, the growth rate ( $r$ ) being determined from the equation

$$r = \frac{\log w_2 - \log w_1}{t},$$

where  $w_1$  and  $w_2$  are the weights of dry matter obtained from successive cuts and  $t$  is the number of days between the sampling dates.

In comparing the calculated values of " $r$ " for the 1931-5 data with the corresponding mean soil temperature (9 a.m. determination at a depth of 4 in.), differences in the rate of manuring from year to year have not been taken into account. Nevertheless, such a comparison does show the general trend of changes with rising temperature in the growth rate of manured and unmanured pasture. Below a temperature of 42° F. no growth takes place. Within a temperature range of 42-44° F. the nitrogen-manured pasture grows at a greater rate than unmanured, the mean value of " $r$ " being 0.0239 as against 0.0091. Within the limits of 44-47° F. the difference is less pronounced, i.e. 0.0257 as against 0.0210. Above 47° F. there is a marked increase in the growth rate, and the value of " $r$ " is the same for manured and unmanured pasture, namely 0.0389 and 0.0385. It can therefore be concluded that the greatest "degree of earliness" will be obtained in those years when the 4-in. soil temperature (at 9 a.m.) rises most slowly from 42 to 47° F., and there is also a considerable period when the temperature ranges between 42 and 44° F. Conversely, with a rapid rise of the soil temperature from 42 to over 47° F., the "degree of earliness" resulting from nitrogenous

manuring may be negligible. The springs of 1930 and 1935 are examples in which the rise in temperature was slow and the "degree of earliness" marked, while the spring of 1934 with its very sudden increase in temperature may be instanced as a year in which the "degree of earliness" was small.

It seems evident from the growth-rate data that while nitrogen is the principal factor controlling the growth of pasture between a soil temperature range of 42–47° F., it is no longer the controlling factor at higher soil temperatures. From this it is concluded that the available nitrogen content of the soil must have increased rapidly with rising temperature due to the greater activity of soil micro-organisms in breaking down organic nitrogen. There seems little doubt that during the winter there is an accumulation of readily nitrifiable material in the form of dead roots, leaves, etc., which can yield on decomposition considerable quantities of inorganic nitrogen. It would appear that below a soil temperature of 47° F. and above 42° F. the rate of decomposition is not rapid enough to supply sufficient nitrogen for the plants to reach the maximum rate of growth possible within that temperature range. In this connexion it is of interest to note that according to Eggleton(5) there is a rapid increase during April in the number of soil microflora in pasture land.

The influence of temperature on the rate of decomposition of organic nitrogen has received little attention from investigators, especially in cases of pasture soils with a high carbon/nitrogen ratio. Schlösing(14) found that nitrification proceeds very slowly between temperatures of 0–5° C. It is clear, however, from Fig. 7 that some accumulation of nitrate can take place below 5° C. It might be suggested that the apparent increase in nitrogen supply with rising temperature may be due in part to an upward movement of the soil solution. With increasing temperature, greater evaporation and transpiration would result, so that nitrogen compounds, leached down by the winter rains, might again be drawn up into the surface layer. While this rise may be a factor in a dry spring such as 1934, it can be of little importance in a season with a normal rainfall in April.

In addition to demonstrating the correlation between nitrogen supply and temperature, these experiments have shown that following upon the application of nitrogenous fertilizers in the early spring, there is a rapid disappearance of the added nitrogen from the soil. These results are in agreement with previous findings(3) for both ammonium sulphate and calcium nitrate applied during the summer months and also with

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the observations of Eggleton<sup>(5)</sup> and Richardson<sup>(13)</sup> on ammonium sulphate. In addition, it has been shown that unexpected fluctuations may occur in the ammonia- and nitrate-nitrogen content of the soil. Out of the three field investigations undertaken in 1931, 1934 and 1935, in no year did the results entirely agree with expectation. In 1931, a week and a fortnight after the addition of sodium nitrate, there was a marked disappearance of nitrate nitrogen, but an unexpected rise in the ammonia-nitrogen content, although this rise cannot be tested for statistical significance. In the third week a sharp and statistically significant increase in the soil nitrate-nitrogen content took place. In 1934 there was a significant increase in the ammonia-nitrogen content of the soil some 8 days after an application of calcium nitrate. Further evidence of this rise in ammonia-nitrogen concentration subsequent to the addition of calcium nitrate was found in 1935.

It is concluded that these fluctuations can be attributed to the action of soil micro-organisms. It is suggested that some of the added nitrate nitrogen is absorbed by certain soil microflora. As the supply of available nitrogen or possibly carbohydrate becomes exhausted, many of these nitrate-assimilating organisms can no longer survive, and the nitrogen in their tissues is broken down into the ammonia form by other organisms. Finally, the ammonia is converted into nitrate if low soil temperature is not inhibiting nitrification. Rapid nitrification may lead to an accumulation of nitrate, and this nitrogen may again be assimilated by the microflora. On such a hypothesis, in 1934, the rise in ammonia nitrogen can be ascribed to the ammonification of the nitrate nitrogen previously assimilated by the microflora. In 1931 the fall and rise in the soil nitrate content can be attributed first to assimilation by organisms followed on their death by the ammonification and nitrification of this absorbed nitrogen. In this connexion it is of interest to note that Eggleton<sup>(5)</sup> has shown at this Station that there is a temporary increase in the numbers of soil microflora following upon the addition of ammonium sulphate to pasture. Furthermore, in a recent paper Burk & Horner<sup>(1)</sup> have demonstrated that ammonia nitrogen may be liberated by *Azotobacter* if the carbohydrate supply becomes exhausted. Under certain conditions this liberation of ammonia nitrogen may be extremely rapid. Moreover, it has been found that cultures raised in a medium containing nitrate nitrogen produced ammonia at a greater rate than those grown previously in ammonia nitrogen. The rises in the ammonia-nitrogen content of the soil observed in the present investigation may therefore be in part due to such excretion of ammonia nitrogen by micro-organisms.

From the findings of this investigation it seems clear that, particularly below a soil temperature of 40° F., nitrification proceeds at a slow rate. The rapid disappearance of ammonia nitrogen from the soil below 37° F. and the simultaneous increase in nitrogen content of the herbage within a few days of the application of ammonium sulphate suggests that the ammonium ions must in part have been absorbed by the roots. Similar evidence of this nature has been brought forward by Richardson (13), who has also studied the changes in the nitrogen content of both the soil and the herbage following upon the application of ammonium sulphate to grassland in the spring. Correlating changes in the total nitrogen content of the herbage with the disappearance of ammonia nitrogen from the soil can only give indirect proof of the absorption of ammonium ions. Direct proof of the absorption of ammonium ions in the field can, however, be obtained by the methods used in this investigation. There seems little reason to doubt that by these methods other plants than the grasses could be shown to absorb ammonium ions.

The importance of temperature and the available nitrogen supply have been stressed in this paper, since it has been shown that the "degree of earliness" obtained by nitrogenous manuring is governed by the length of time that the soil temperature remains within a range of 42 and 47° F. Failure to obtain an "early bite" can, however, be due to other causes than a rapid rise in temperature from 42 to 47° F. The investigations of Martin Jones (8, 9) indicate that the response of pasture to nitrogenous manuring in the spring is related to the previous grazing treatment. Hard grazing in the autumn and winter weakens the grasses, while, conversely, "resting" the pasture leads to the largest responses. In addition, the botanical composition of the pasture is of importance. All the investigations cited have been carried out on mixed swards, where differences in reaction between species are masked both in regard to changes in temperature and nitrogen supply. Observations on plots of different species indicate that *Lolium perenne* (perennial rye grass) grows earlier in the season and responds more quickly to nitrogenous manuring than the majority of other species. The "maximum degree of earliness" will therefore be obtained from pastures which contain a high proportion of *L. perenne*, have been rested during the autumn and winter months, and have received a fairly heavy application of nitrogenous fertilizer. The apparent capriciousness of the "early bite" effect is thus readily understood. It is believed that cases of failure to obtain an "early bite", e.g. as reported by Woodman & Underwood (16), can be explained if due



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regard is paid to the circumstances of soil temperature, nitrogen supply, botanical composition, and previous grazing or cutting treatments.

Finally, while these investigations have been confined to a study of the growth of pasture in the spring, it is probable that apart from low temperature, the available nitrogen supply is a factor controlling the growth of other species. It is suggested that plants able to make growth in the winter or early spring must have either low nitrogen requirements or reserves of nitrogen as well as carbohydrates stored in the tissues.

### SUMMARY

A study extending over 6 years (1930-5) has been made of the principal factors controlling the growth of pasture in the spring. Replicated experiments were carried out on a number of pastures varying in botanical composition. It has been found that:

(i) Pasture dressed with nitrogenous fertilizers in February or early March and first harvested in early May, gave very variable increases in yield from year to year.

(ii) In 5 out of 6 years the changes in the growth rate were determined by cutting at frequent intervals a succession of plots. Below a soil temperature at 4 in. of approximately 42° F. no growth took place. Between 42 and 47° F. nitrogen-manured pasture grew at a greater rate than the control, but at higher temperatures the growth rates were very similar. The manured herbage reached the grazing stage sooner than did the control; the "degree of earliness" varied from 13 to 14 days in 1930 and 1935, to 2 days in 1934.

(iii) Nitrogenous fertilizers brought about marked changes in the nitrogen content of the herbage; in general, a maximum value (3.9-5.3 per cent) was reached a week or 10 days after growth commenced. Significant increases in the nitrogen content were obtained within 4-8 days of the fertilizer application, even at soil temperatures below 37° F., when no growth was occurring. The absorbed nitrogen was not immediately synthesized into protein. An application of calcium nitrate led only to an accumulation of nitrate nitrogen, while ammonium sulphate brought about increases in the amide and ammonia nitrogen, but no increase in the nitrate fraction. Such differences can be ascribed only to the direct absorption of ammonium ions by the roots.

(iv) In 4 years the ammonia- and nitrate-nitrogen content of the soil showed that within 10 days a large percentage of the added nitrogen could not be recovered from the soil in an inorganic form. Certain anomalous fluctuations in the concentration of ammonia and nitrate

nitrogen were observed, notably a statistically significant increase in the ammonia nitrogen following upon the addition of calcium nitrate.

From these results it is concluded that below 42° F. temperature is the factor controlling growth. Over a temperature range of 42–47° F., in the absence of manuring, growth is limited by the rate at which inorganic nitrogen is liberated by soil micro-organisms. At higher temperatures the rate of liberation increases rapidly, and nitrogen supply no longer controls growth. It follows, therefore, that the greatest response to nitrogenous manuring may be expected in a year when the soil temperature rises slowly from 42 to 47° F., and conversely the smallest response when the rise between these temperatures is rapid.

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# COTTON-SEED TREATMENT WITH SULPHURIC ACID

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(With Plate V)

## INTRODUCTORY

As early as 1911 Duggar & Canther<sup>(5)</sup> used commercial sulphuric acid for controlling cotton anthracnose in Alabama. Their method consisted in placing the cotton seed in a wooden or earthen vessel and then covering it with commercial sulphuric acid for about 10 min. During the process the seed was constantly stirred and, after the acid was drained, it was thoroughly washed in running water and dried.

Since then several workers have studied the effect of this treatment upon the cotton seed not only with regard to checking cotton diseases transmitted through the seed, but also in connexion with other possible advantages, which might have resulted by this process.

The efficiency of sulphuric acid in killing surface parasites of the cotton seed, with which this paper is not concerned, has been reported by several authors<sup>(4, 5, 6, 7, 10, 11, 12)</sup>. Moreover, it has been shown that the elimination of the short hairs (fuzz) surrounding the cotton seed by the sulphuric acid method resulted, in comparison with untreated seed, in: (1) earlier germination<sup>(3, 4, 6, 10, 11)</sup>, (2) higher percentage of seeds germinating<sup>(3, 4, 9, 10)</sup>, (3) higher yield of seed cotton per acre<sup>(2, 3, 6)</sup> and (4) better and more uniform planting by machine<sup>(3, 4, 10, 12)</sup>. It should be noticed that some of the above advantages are mentioned<sup>(1)</sup> even in the case of seed delinted by mechanical process in the ginneries.

Concentrated sulphuric acid was mostly used in delinting cotton seed and 5–15 min. were advocated as the optimum duration of the treatment<sup>(3, 5, 8)</sup>. However, according to some evidence<sup>(9, 11)</sup>, much longer duration (up to 4 hours) did not cause any damage to the seed. A higher strength of sulphuric acid was found less injurious to the germinative value of the cotton seed, although a new method has been suggested<sup>(8)</sup>, by which diluted acid (1 part of the acid to 5 parts of water) may be used profitably, the whole process lasting, in this case, for 1–2 weeks.

The method originally adopted by Duggar for the application of the sulphuric acid treatment is very slow, tedious, expensive and dangerous (on account of the handling of concentrated sulphuric acid in open vessels). However, in 1925 an appropriate machine was designed (4), which can deal safely and economically with large quantities of cotton seed.

The above-mentioned papers, which deal with the importance of the sulphuric acid treatment of the cotton seed, do not provide in the majority of cases sufficient arithmetical data to prove that the effect of this treatment is such as it is often assumed to be. Even in cases where experimental data are available, they do not lend themselves to proper statistical analysis, so that their significance is most uncertain in the light of modern experimental technique. Therefore, in order to show whether treating the seed with sulphuric acid should be recommended or encouraged for general use, it was first necessary to investigate whether the advantages advocated in this respect are supported by adequate experimental data.

The following questions have been studied in connexion with the sulphuric acid treatment of the cotton seed:

- I. Rate of germination.
- II. Earliness of germination.
- III. Yield per acre.
- IV. Lint percentage.
- V. Better and more uniform planting.
- VI. Loss in the weight of seed.
- // VII. Concentration of the sulphuric acid employed.
- // VIII. Effect of the length of time after the treatment.

In this respect several experiments have been carried out at the Greek Cotton Institute (Central and Substation) during the years 1932-5. In most cases preliminary investigations were necessary for studying the best means of keeping under control the various factors affecting the experimental data. Owing to the enormous amount of figures involved, averages are usually given in this paper with their statistical significance.

## I. RATE OF GERMINATION

### *Material and methods*

The tests for determining whether the sulphuric acid treatment has any effect on the rate of germination were carried out both in the laboratory and in the field. The seed used was produced at the Central

Experimental Farm of the Institute at Sindos in 1934. Only first pickings were employed, and for these experiments the following five varieties were chosen as belonging to several species and representing various conditions of seed (Pl. V):

- (1) Serres (local), fuzzy—*G. hirsutum*.
- (2) Serres (local), black—*G. hirsutum*.
- (3) Zagora (Egyptian), black—*G. Barbadosense-Peruvianum*.
- (4) Acala (American), fuzzy—*G. hirsutum*.
- (5) Batouto (local), fuzzy—*G. herbaceum*.

From each variety a lot of about 3 kg. of seed was taken and, after being made as uniform as possible, samples of 175 g. each were separated. In making these samples particular care was taken to secure uniformity, nine samples of 175 g. each being formed from each of the above five varieties.

For treating the seed with sulphuric acid nine time-intervals were adopted: 0, 5, 15, 25 and 40 min., 1, 2, 5 and 12 hr. The quantity of concentrated sulphuric acid (65·9° Beaumé of specific gravity 1·84) used for each sample of 175 g. of seed, was 300 c.c. The seed and the acid were placed in appropriate glasses, the stirring being uniform for each sample (during the first 5 min. the seed was stirred continuously, and then the stirring was repeated regularly at every quarter of an hour). The work was so arranged for each variety that the treatment with the reagent was always finished at about the same time (between 7.30 and 8 o'clock in the evening). After the treatment each sample was thoroughly washed in running water and dried in the room at ordinary temperature (18–20° C.); the proper germination test beginning at noon of the following day.

#### *Laboratory tests*

It should be noticed that the laboratory germination tests with cotton seeds are extremely delicate. The percentage of seeds germinating as well as the time required for the appearance of the radicles vary enormously according to the maceration with water or otherwise of the cotton seed previous to putting it in the water bath, to the temperature and the ventilation of the incubator, to the use or otherwise of glass bell-jars and cotton or woollen mats, to the number, thickness and quality of the filter papers used, etc.

With each of the previously mentioned five cotton varieties, five tests in all were carried out under similar experimental conditions. For each treatment nine samples of fifty seeds were used, arranged according to

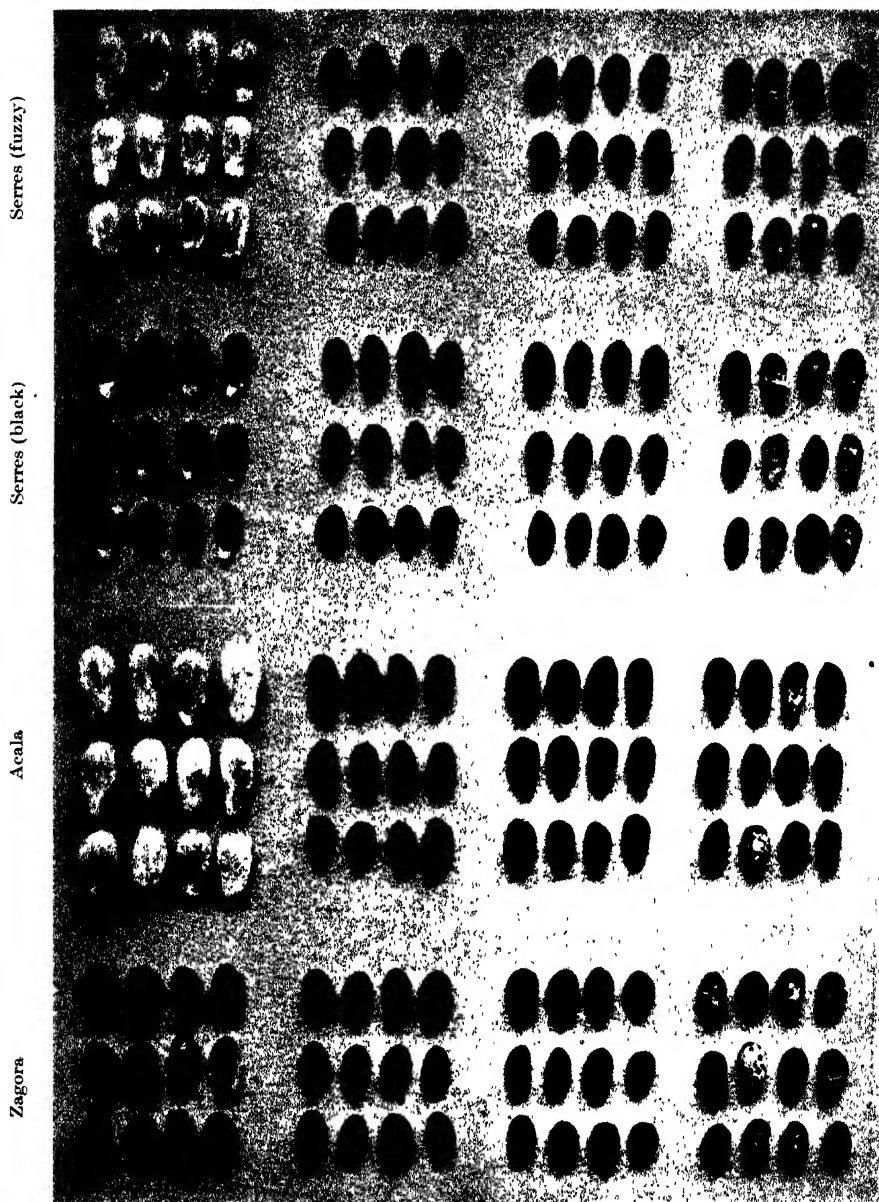


Not treated

1 hr.

5 hr

12 hr.



Seeds of four cotton varieties untreated or treated with commercial sulphuric acid for 1, 5 and 12 hours.

a Latin Square arrangement in an electric Copenhagen germinator (with automatic temperature regulator). In order to save space the analytical data are not given here. Table I, however, contains the mean percentage of germination for all varieties and treatments, as well as the analysis of variance for the complete series of data provided by the five varieties.

Table I. *Effect of the sulphuric acid treatment on the percentage of germination in five varieties of cotton: laboratory tests*

Time interval of the treatment	Percentage of germination for each variety					Average for treatment
	Serres (fuzzy)	Serres (black)	Zagora	Acala	Batouto	
12 hr.	73.8	88.4	74.2	70.4	74.4	76.07
5 hr.	79.8	90.0	75.8	93.3	85.3	84.84
2 hr.	98.4	98.2	91.6	97.6	88.9	94.93
1 hr.	97.1	96.4	92.7	96.2	88.2	94.13
40 min.	97.1	97.1	95.6	97.1	89.3	95.24
25 min.	95.8	97.8	92.7	95.3	86.9	93.69
15 min.	96.4	97.8	94.2	98.7	90.7	95.55
5 min.	95.8	98.0	89.1	94.7	88.9	93.29
0 min.	95.8	98.0	71.8	93.6	85.3	88.89
Difference statistically significant	5.12					5.44
Average for varieties	92.22	95.76	86.40	93.98	86.44	
Difference statistically significant	4.48					

### *Analysis of variance*

	D.F.	S.S.	Var.	S.D.	log <sub>e</sub> S.D.
Between varieties 4	4	1411.59	352.90	18.79	2.93339 (1)
Within varieties $80 \times 5 = 400$					
(a) Between rows	8	153.31	19.16	4.38	1.47705 (2)
Inter. rows 5 varieties	32	337.81	10.56	3.25	1.17865 (3)
(b) Between columns	8	227.60	28.45	5.30	1.66771 (4)
Inter. col. 5 varieties	32	490.74	15.34	3.92	1.36609 (5)
(c) Between treatments	8	3773.57	471.69	21.72	3.07824 (6)
Inter. tr. 5 varieties	32	1330.52	41.57	6.45	1.86408 (7)
(d) Error $56 \times 5$	280	2052.30	7.33	2.71	0.99695 (8)
Total	404				

$z(1-7) = 1.06931$  (for  $n_1 = 4$ ,  $n_2 = 30$  and  $P = 5\%$ ,  $z = 0.4947$ )  
 $z(2-3) = 0.29840$  (for  $n_1 = 8$ ,  $n_2 = 30$  and  $P = 5\%$ ,  $z = 0.4090$ )  
 $z(4-5) = 0.30162$  (for  $n_1 = 8$ ,  $n_2 = 30$  and  $P = 5\%$ ,  $z = 0.4090$ )  
 $z(6-8) = 2.08129$   
 $z(7-8) = 0.86713$  } greatly significant

The data point to the conclusion that in several cases there exists a significant difference, with regard to the percentage of germination, between the five varieties tested, in spite of the fact that the seeds were produced, stored and tested under as much the same conditions as practically possible. Moreover the seed treated with commercial sul-



phuric acid from 0 min. to 2 hr. and even to 5 hr. with some varieties, does not show any decrease in the rate of germination. On the contrary, in one case (the Egyptian variety Zagora), the non-treated seed or that treated only for 5 min. germinated considerably less than seed treated with the acid for from 15 min. to 2 hr. However, in the case of a 12 hr. treatment a decline in the rate of germination is revealed. It should be noticed that the effect of the treatment is much more intense with the Zagora than with other varieties of cotton. Also that the variance ascribed to rows and columns does not reach the level of significance.

### *Field tests*

The germination tests in the field were carried out with the same seed as that used in the laboratory. Only three varieties, i.e. Serres (fuzzy), Acala and Zagora were studied owing to the difficulties involved in carrying out detailed germination tests under field conditions.

The seeds were planted in small rows 3 m. long and 20 cm. apart, the distances in the row between the seeds being 5 cm. (For every six rows there was a path 40 cm. wide for counting the germinating seeds.) Samples of fifty seeds were again adopted and sown each in one row. For every experiment, covering one variety, the nine treatments of seed were repeated nine times as usual.

In order to plant the seeds at a uniform depth a dibbing iron was made use of. The holes were 3 cm. deep, and after one seed was put in each hole, they were filled up with a mixture of sand and farm-yard manure to avoid crust formation on the surface. When necessary water was given by means of a hand-sprinkler. The test was designed according to the randomized block system. A seed was counted as germinating when the cotyledons were visible above the ground.

As would be expected the field data present greater variability in comparison with those found in the laboratory. The coefficients of variation for the percentage of germination in both cases were as follows:

Variety	Coefficient of variation (s.d. % of the mean)	
	Laboratory data	Field data
Serres (fuzzy)	3.4	14.3
Acala (pedigree seed)	3.6	12.6
Zagora	12.4	23.1

It was not possible to carry out all three tests at the same time. The first test with the Serres (fuzzy) variety started on 13 April and lasted until 5 May (average temperature for the whole period 13.9° C.). The second test (variety Acala) lasted from 7-21 May (average temperature

17.6° C.). Finally the Zagora experiment started on 27 June and was over on 13 July (average temperature 21.7° C.).

The mean percentage of germination obtained for every one of the above three varieties and each treatment are given in Table II together with their statistical significance. It should be observed that the results obtained are more or less in agreement with those reached in the laboratory. The only appreciable difference is that the seeds given 12 and 5 hr. treatments practically do not germinate at all, while the 2 hr. and the 0 min. treatment show a definite decline in germination. Moreover, the data obtained from the field tests show a general fall in the germinative capacity of the seed. This in connexion with the previous observation shows that seeds, the embryos of which are completely healthy, may not germinate under field conditions. Therefore the indications of laboratory incubators do not represent the practical germination value of seeds even when particular precautions are taken in this respect.

Table II. *Effect of the sulphuric acid treatment on the percentage of germination: field tests*

Time interval of the treatment	Percentage of germination for each variety			Average for treatments
	Serres (fuzzy)	Acala	Zagora	
12 hr.	0.2	3.8	2.4	2.1
5 hr.	4.9	24.4	12.4	13.9
2 hr.	49.1	66.7	60.8	58.9
1 hr.	63.6	77.8	70.0	70.5
40 min.	60.9	75.6	60.0	65.5
25 min.	70.4	74.2	62.4	69.0
15 min.	61.1	72.4	65.2	66.2
5 min.	63.6	73.6	64.4	67.2
0 min.	55.1	72.2	58.4	61.9
Difference statistically significant	10.96			6.33
Average for varieties	47.6	60.1	50.7	

## II. EARLINESS OF GERMINATION

The data provided by the experiments mentioned in the preceding paragraphs were also used for the determination of the earliness of germination.

With regard to the laboratory tests the mean time required for germination was worked out from the daily records for every sample of fifty seeds. The means so obtained (81 for each variety or 405 in all) were analysed according to the analysis of variance method, the results given in Table III.

It is obvious that the sulphuric acid treatment had a considerable

effect on the earliness of germination. For all the varieties tested the untreated seed requires a much longer time for germination than the seed treated with the reagent for 5 min. or more. Between 15 min. and 2 hr. there is not any appreciable difference in the time of germination, whereas the 5 hr. treatment germinates later than the shorter treatments, and curiously enough later than even the 12 hr. treatment in most cases.

Table III. *Effect of the sulphuric acid treatment on the earliness of germination: laboratory tests*

Time interval of the treatment	Mean time of germination in hours					Average for treatment
	Serres (fuzzy)	Serres (black)	Zagora	Acala	Batouto	
12 hr.	64.1	54.5	43.3	49.1	32.8	48.8
5 hr.	69.3	59.4	52.9	45.3	31.5	51.7
2 hr.	41.2	36.0	34.5	28.3	23.4	32.7
1 hr.	40.6	30.7	32.5	31.5	24.9	32.0
40 min.	37.2	30.9	33.9	30.9	25.1	31.6
25 min.	36.3	30.3	30.9	32.7	25.3	31.1
15 min.	42.1	38.8	34.3	31.3	26.1	34.5
5 min.	58.6	62.4	78.7	51.0	46.7	59.5
0 min.	77.7	100.1	133.0	86.2	75.3	94.5
Difference statistically significant			5.02			11.78
Average for varieties	51.9	49.2	52.7	42.9	34.6	
Difference statistically significant			8.78			

It should be noticed, however, that the earliness of germination depends largely on the amount of moisture available. The untreated seed, for example, germinates much more quickly when it is soaked in water for a few minutes before being put in the incubator than otherwise, whereas for seed treated with sulphuric acid this does not make any appreciable difference. (It seems that the water provided through the strips of filter paper, while ample for the treated seed, is not enough for the germination of untreated seed.) In preliminary experiments, where the seeds were first soaked in water, the differences in time of germination were much smaller, although still significant, than in the final tests (the data of which are recorded in Table III), where the seeds were dry. Nevertheless, the latter procedure was finally adopted in order to equalize the quantity of water given to the various treatments of seed. On the other hand, the effect of the sulphuric acid treatment on the earliness of germination does not depend on the existence of fuzz, since the fuzzless varieties, Serres black and Zagora, are more affected by such treatment than the other fuzzy varieties.

The field data are not as conclusive as the laboratory data, presum-

ably owing to the variations in soil moisture. Table IV contains the mean number of days required for germination provided by three experiments carried out under field conditions. Only two of them gave results statistically significant, from which it is shown that in agreement with the laboratory data the untreated seed requires a longer time for germination than the treated seed. (The 5 and 12 hr. data are not included because the germination of the respective seeds was exceedingly poor.) These results correspond to those obtained at the laboratory with seeds soaked in water, since there is only a slightly earlier germination with the treated seed amounting to a few hours or about 10 per cent of the mean germination period. Moreover, the differences existing between the three varieties tested cannot be exclusively attributed to variety, since the temperature and moisture conditions were not the same for the three experiments.

Table IV. *Effect of the sulphuric acid treatment on the earliness of germination: field tests*

Time interval of the treatment	Mean time of germination in days			
	Serres (fuzzy)	Acala	Zagora	Average
2 hr.	13.2	8.8	9.1	10.4
1 hr.	12.9	8.6	8.5	10.0
40 min.	13.3	8.7	9.1	10.4
25 min.	12.9	8.8	9.3	10.3
15 min.	13.2	8.9	8.8	10.3
5 min.	12.9	8.7	9.0	10.2
0 min.	13.7	9.4	9.6	10.9
Difference statistically significant	0.5	0.3	Not significant	
Date of planting	13. iv. 35	7. v. 35	27. vi. 35	
Date of the beginning of germination	23. iv. 35	11. v. 35	2. vii. 35	

In conclusion it is justifiable to assume that in cases where the amount of moisture available is limited, as in the case of laboratory tests with dry seeds, treated seeds germinate considerably earlier than those not treated. If, however, the moisture is sufficient, as happened in the field tests or in the laboratory with soaked seeds, the difference in earliness between treated and untreated seeds is comparatively small.

### III. YIELD PER ACRE

In 1933, 1934 and 1935 several experiments were carried out to determine whether the use of cotton seed treated with sulphuric acid had any appreciable effect on the yield per acre. These experiments involved

several varieties and were carried out in two places in Macedonia according to the randomized block system.

The delinting was done in the following manner:

A sufficient quantity of the acid (65.9° Beaumé) was poured over the seed to thoroughly mix with it. The seed was stirred for about 10-15 min., after which the acid was drained and the seed completely washed in water and dried. Particular care was always taken to secure that the two lots of seed, one of which was untreated and the other treated with the reagent, were as uniform as possible.

At sowing time the seed was planted in hills 20–30 cm. apart by means of the Cole no. 40 cotton planter. The distances between the rows varied from 0.80–1.00 m. Three-row plots were always used, except in 1934, when single-row plots were laid out. In 1933 all three rows were taken into account, whereas in 1935 only the middle row was considered in order to avoid any competitive effect.

The data obtained are summarized in Table V, where the means for each variety and each experiment are given separately, together with the

Table V. *Average yield per plot (in kg.) with several varieties of cotton and seed treated (T.) or untreated (U.) with sulphuric acid*

[illegible]

results of their statistical analysis. The respective sizes of plots as well as the number of replications are also included. It should be mentioned that Sindos is situated just outside Salonica in the Axios plain. Its soil consists of deep sandy loam. Verria is about 60 km. away, lies between 80 and 90 m. above sea-level and its soil is clay rich in organic matter with a hard calcareous subsoil, reaching at places to a very short distance from the surface (40 cm.).

The figures given in Table V indicate that only in one case (Sindos 1933, with the Egyptian varieties), did the result appear to approach significance in favour of treated seed, which yielded about 5.7 per cent more than the untreated one. In all other cases the use of either seed was of no consequence, showing that in so far as the yield per acre is concerned, the farmer does not benefit by using seed delinted with sulphuric acid.

#### IV. LINT PERCENTAGE

Lint percentage, or the amount of lint obtained from 100 lb. of seed cotton, constitutes an important economic factor in cotton growing. Therefore, the data of the previous chapter have been used to find out whether delinting the seed by sulphuric acid has any effect on the lint percentage. Since the crop from every plot was picked, ginned and weighed separately, the ginning out-turn has been worked out for every

Table VI. *Average lint percentage with several varieties of cotton and seed treated (T.) or untreated (U.) with sulphuric acid*

Varieties	Locality and year of the experiments											
	Sindos 1933		Sindos 1933		Sindos 1934		Verria 1934		Sindos 1935		Verria 1935	
	U.	T.	U.	T.	U.	T.	U.	T.	U.	T.	U.	T.
Serres	27.7	27.4	28.7	28.9	28.1	28.3	30.6	30.6	28.4	28.4	29.0	29.3
Ingold	31.7	31.2	—	—	—	—	34.4	34.3	—	—	—	—
Trice	27.9	28.0	—	—	—	—	32.1	32.1	—	—	—	—
Acala	30.9	32.5	—	—	—	—	33.8	33.1	—	—	—	—
Carolina Foster	28.1	28.4	—	—	—	—	—	—	—	—	—	—
Delfos	26.8	28.2	—	—	—	—	—	—	—	—	—	—
Cleveland	30.1	30.2	—	—	—	—	—	—	—	—	—	—
Wilds	27.6	27.2	—	—	—	—	—	—	—	—	—	—
Cook	31.7	29.9	—	—	—	—	—	—	—	—	—	—
Sunshine	—	—	—	—	—	—	—	—	—	—	—	—
King	—	—	—	—	—	—	30.8	30.2	—	—	—	—
Tsitairos	—	—	—	—	—	—	—	—	31.8	32.0	31.9	31.7
Pelion	—	—	31.7	31.7	33.5	33.2	—	—	—	—	—	—
Nahda	—	—	30.6	30.5	31.0	31.2	—	—	—	—	—	—
Fouadi	—	—	30.4	30.2	30.7	30.5	—	—	—	—	—	—
Sakellaridis	—	—	28.4	28.6	29.3	29.3	—	—	30.9	30.7	32.2	32.1
Sakha	—	—	30.0	29.8	—	—	—	—	—	—	—	—
Average	29.2	29.2	30.0	30.0	30.5	30.5	32.3	32.1	30.4	30.4	31.0	31.0

plot and every experiment, and the results summarized in Table VI. It is obvious that the sulphuric acid treatment has no effect on the percentage of lint.

Moreover the quality of lint has been examined and, so far as it could be determined by the grader, no difference in the quality of cotton or the hair-length could be established between the produce of treated or untreated seed.

#### V. BETTER AND MORE UNIFORM PLANTING

Naked seed, as it is obtained after treating the seed with sulphuric acid, is easier to handle than fuzzy seed. At the same time the use of a seed-cleaner is greatly facilitated in the first instance, whereas in the latter the cleaning of the seed is often defective, or even impossible owing to the existence of fuzz. Finally it is probable that with some types of planters sowing naked seed gives a better and more uniform stand of cotton.

It should be noticed, however, that with an appropriate planter (like Cole no. 40), the work is equally satisfactory with either seed. In the last three years, during which treated and untreated seeds were sown for comparison in adjacent plots, any difference in this respect has scarcely been observed. Nevertheless, it should be borne in mind that treated seed falls more freely through the holes of the planter's ring than fuzzy seed. Therefore when using the same ring with both kinds of seed (treated and untreated), any difference in the stand of cotton may be attributed to the difference in the amount of seed employed. On the other hand, it should not be lost sight of that when the amount of soil moisture is limited, treated seed may germinate earlier and better than the untreated.

#### VI. LOSS IN WEIGHT OF SEED

In order to determine what was the loss in weight of seed after its treatment with sulphuric acid, samples of seeds of about 20 g. each were subjected to the effect of the reagent for various intervals of time. The acid used was of 65.7° Beaumé (specific gravity 1.834). The seeds after the treatment were dried in an ordinary heated room until their weight remained constant. The weighings were done by means of a chemical balance. Two experiments of the same type with five replications each were carried out, one with fuzzy and the other with black seed of the local variety Serres. The means and the statistical differences for both experiments are shown in Table VII.

Table VII. *Mean loss in weight of seed*

Time interval of the treatment	Mean percentage decrease in weight	
	Black seed	Fuzzy seed
5 min.	4.16	7.50
15 min.	6.06	9.20
25 min.	7.23	9.57
40 min.	8.05	9.87
1 hr.	8.27	11.32
2 hr.	10.70	13.38
5 hr.	13.35	16.57
12 hr.	20.88	22.19
Statistically significant differences between any two treatments of the same seed ( $P=5\%$ )	$\pm 0.40\%$	$\pm 0.69\%$

The data, being particularly conclusive in this respect, show that seed treated with commercial sulphuric acid loses from 4 to more than 20 per cent of its weight, according to the kind of seed and the time interval of the treatment. The same conclusion more or less was also reached by the figures provided by the experiments of Part I, where the seed before and after the treatment was weighed for every variety or time interval.

## VII. CONCENTRATION OF THE SULPHURIC ACID EMPLOYED

Concentrated sulphuric acid is mostly recommended for delinting cotton seed, because it is supposed to be more effective and less injurious to the germination of the seed. Sherbakoff(s), however, suggested the use of acid considerably diluted with water. In order to determine what is the effect of various strengths of sulphuric acid, a series of tests was carried out with solutions in water of 75, 50, 25 and 10 per cent of the acid. Preliminary trials had shown that when using acid of various strengths, the seed fuzz was removed in the following intervals of time:

Acid 10 per cent in 14 days  
 25     ,,     in 7 days  
 50     ,,     in 24 hr.  
 75     ,,     in less than 30 min.

Concentrated in less than 5 min.

Therefore, when planning the present experimental work, for each strength of acid the intervals of time adopted were so chosen that they extended above as well as below those indicated for the removal of fuzz.

The same methods and the same precautions adopted in the case of concentrated sulphuric acid, were also observed with the diluted reagent. The tests were carried out in the laboratory with the Serres (local)



variety of seed (a commercial mixture of black and fuzzy seeds) and by means of the same Copenhagen seed germinator, using nine samples of fifty seeds each for every treatment. The results obtained with regard to the rate and earliness of germination are summarized in Table VIII which contains the means for each treatment, as well as their statistical significance.

It is noticeable that, as the acid employed is more diluted with water, the seed can stand its effect for longer periods of time without losing its germinative value. At the same time with diluted acid the seed germinates earlier with longer time intervals of treatment, as is shown in the following table.

Time intervals of treatment

Acid employed %	During which no decrease in the rate of germination is taking place	Corresponding to the earliest germination amongst all intervals tested
Concentrated	0 min.—2 hr.	25 min.—1 hr.
75	0 min.—12 hr.	3 hr.—24 hr.
50	0 min.—24 hr.	12 hr.
25	0 min.—4 days	2—10 days
10	0 min.—under 3 days	14—28 days

In the case of a 10 per cent solution the seed becomes mouldy owing to the considerable length of the process.

The above results are in close agreement with those obtained in the

Table VIII. *Mean percentage and mean time of germination as affected by the use of diluted acid (75, 50, 25 and 10 per cent of acid in water)*

Time interval of the treatment	Mean percentage of germination				Mean time of germination in hours			
	75%	50%	25%	10%	75%	50%	25%	10%
0 min.	95.6	95.6	95.3	95.3	59.0	59.0	63.4	63.4
30 min.	97.6	—	—	—	21.9	—	—	—
3 hr.	97.1	—	—	—	19.0	—	—	—
6 hr.	—	97.6	—	—	—	25.7	—	—
12 hr.	96.9	97.6	—	—	18.9	21.0	—	—
1 day	92.2	95.8	96.4	—	19.5	23.5	39.0	—
2 days	—	82.9	94.7	—	—	22.6	31.7	—
3 days	—	—	—	79.1	—	—	—	48.7
4 days	—	—	92.4	—	—	—	29.3	—
5 days	—	—	—	67.1	—	—	—	45.4
7 days	—	—	68.7	—	—	—	32.5	—
10 days	—	—	52.0	—	—	—	26.9	—
14 days	—	—	36.7	48.4	—	—	40.1	40.6
18 days	—	—	10.0	—	—	—	46.0	—
21 days	—	—	—	38.2	—	—	—	39.1
28 days	—	—	—	14.4	—	—	—	34.5
Differences statistically significant	±3.0	±3.0	±8.24	±8.24	±1.48	±1.48	±6.4	±6.4

case of the concentrated reagent. Cotton seed treated with sulphuric acid for several hours or even days (depending on the concentration of the acid) retains its germination value unaltered. Besides it shows a remarkable earliness in germination, which with the use of more and more diluted acid is most pronounced with longer intervals of treatment.

# VIII. EFFECT OF THE LENGTH OF TIME AFTER THE TREATMENT

The germination tests referred to in Parts I and II above were carried out immediately after the treatment of the seed as soon as it had been washed in water and dried. However, it is important to know whether delinted seed tested some time later would retain its germinative value, as well as the earliness in germination observed with treated seed. Accordingly samples of the remaining seed of the Serres black variety (the same as that used for the experiments of Parts I and II), were tested again 6 months after the treatment, in precisely the same way as the earlier tests. The data obtained are given in Table IX.

Table IX. *Rate and earliness of germination six months after the treatment*

Time interval of the treatment	Percentage of germination		Mean time of germination in hours	
	Immediately after the treatment	6 months later	Immediately after the treatment	6 months later
12 hr.	88.4	64.9	54.5	28.5
5 hr.	90.0	68.4	59.4	36.4
2 hr.	98.2	98.2	36.0	22.3
1 hr.	96.4	96.4	30.7	20.6
40 min.	97.1	98.9	30.9	20.4
25 min.	97.8	99.3	30.3	20.4
15 min.	97.8	98.9	38.8	24.7
5 min.	98.0	98.7	62.4	35.3
0 min.	98.0	98.2	100.1	54.1
Differences statistically significant	±3.2	±6.4	±4.3	±1.7

The figures of Table IX show that only the 12 and 5 hr. treatment presented a considerable decrease in the rate of germination, which is apparently due to an excessive growth of moulds, developed some 24 hours after the beginning of the test. The seed subjected to the other treatments remained practically unaltered, indicating that it retains the same germinative value for a long period after its treatment with the acid.

With regard to the earliness in germination it is noticeable from the figures of the 0 min. treatment that cotton seed germinates considerably

earlier, several months after the picking has taken place, than when it has just been picked. Nevertheless in so far as the different treatments are concerned, their comparative earliness in germination is the same in both cases (immediately after the treatment or six months later) the 25 min., 40 min. and 1 hr. treatments being in both significantly earlier than the rest.

#### SUMMARY AND CONCLUSIONS

1. Several authors have previously reported that the sulphuric acid treatment of the cotton seed resulted in: (a) earlier germination, (b) higher percentage of seeds germinating, (c) higher yield of seed cotton per acre, and (d) better and more uniform planting (this paper is not concerned with the disinfecting effect of this process).

2. A series of experiments, both in the laboratory and under field conditions have been carried out to determine whether the above statements are in agreement with actual experimental data.

3. In so far as the rate of germination is concerned, it was found that: (a) untreated seeds often show a smaller percentage of germination than seeds treated with sulphuric acid for various time-intervals, particularly when tested in the laboratory; (b) cotton seed treated with the reagent for from 5 min. to 2 hr. or even 5 hr. in some cases, does not show any decrease in its germinative value as determined by laboratory tests. In field trials seeds treated for 5 or 12 hr. practically do not germinate at all, whereas the 2 hr. treatment often shows a small decline in germination; (c) an enormous difference in the rate of germination has been revealed between the laboratory determinations on the one hand and the field determinations on the other.

4. There is an unmistakeable earliness in the germination of the treated seed. When during the germination period the moisture available is limited, the earliness is considerable as happens in laboratory tests with dry seeds. In cases, however, where the moisture is sufficient the earliness of the treated seed is so small that it does not seem to be of any particular consequence (this is the case in laboratory tests with seeds previously soaked in water for a few minutes, or in the field tests considered).

5. The data of several experiments involving many cotton varieties and carried out for three successive years, point to the conclusion that the yield per acre as well as the ginning out-turn do not seem to be affected by the use of either treated or untreated seed.

6. Delinted seed is easier to handle, as well as to clean by a seed

cleaner. Also it is better sown by some types of planters, although any difference in this respect may often be attributed to the fact that delinted seed falls more freely through the holes of the planter's ring.

7. The weight of seed treated with sulphuric acid decreases appreciably according to the time interval of the treatment and the seed being covered or otherwise with fuzz. The loss in weight in the cases considered amounted to from 4 to more than 20 per cent of the original weight.

8. The use of diluted acid has presumably similar effects to those caused by concentrated acid. However, these effects are delayed, according to the degree of concentration.

9. Seeds tested just after their treatment with the acid or 6 months later, showed in both cases the same germinative value. In the latter case, however, the seed on the whole germinated considerably earlier than in the former, whereas the comparative differences between the various time intervals of treatment remained almost unaltered.

10. The experimental evidence discussed in this paper does not seem to justify the use of seed treated with sulphuric acid, since no practical advantage attached to it could be definitely established.

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# THE BASE STATUS OF SCOTTISH SOILS

## I. THE EFFECTS OF LIME ON FIVE TYPICAL SOILS FROM NORTH-EAST SCOTLAND

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(With Six Text-figures)

IN order to study the effects of lime on soils from the north-east of Scotland a series of laboratory and field experiments was carried out on five representative areas. The selection was made on a geological basis, with the restriction that the soils should be moderately acid in reaction. At each centre a set of eighteen equal plots, in six rows of three plots, was laid down on an area of one-half to one acre. Six of the plots received a dressing of slaked lime equivalent to 1 ton CaO per acre and a further six plots double this amount. The lime was applied to the ploughed land and subsequently harrowed in. Representative samples to a depth of 9 in. were drawn by soil auger in March 1932, previous to the application of the lime, and subsequently at 6 months and 1 year after liming.

The pH values were determined on the moist samples from individual plots, after which the soils were air-dried and passed through a 2 mm. sieve. All subsequent determinations were made on composite samples from plots receiving similar treatment.

Oats were grown during the first year. In the first four areas (Table I) a grass-clover mixture was sown with the oats. The fifth area was fallowed over the winter and cropped in turnips in the succeeding year. The lime applied contained 85.7 per cent  $\text{Ca(OH)}_2$  and 7.6 per cent  $\text{Mg(OH)}_2$ . The dressings were equivalent to 0.95 and 1.91 tons of calcium oxide per acre, with a Ca : Mg ratio of 8.86.

The soils selected for investigation are characterized by the data contained in Tables I, II and III. Table I gives the location and geological origin of the soils, which are referred to throughout the text by the name of the farm on which the plots were situated. The annual rainfall at each locality is 30–35 in., evenly distributed over summer and winter. The weather of the period under investigation was normal in this respect.

Tables II and III give the mechanical and ultimate chemical compositions of the fine material, together with a volume weight determination.

Table I. *General characterization of the soils*

Soil	Parent material	Description of surface 9 in.	Locality
Overhill	Boulder till, containing basic and acidic rock types, overlying epidiorite	Dark brown medium loam	Overhill, Belhelvie, Aberdeenshire
Drumnagair	Boulder till derived mainly from local old red sandstone rocks	Reddish brown heavy loam	Drumnagair, Laurencekirk, Kincardineshire
Inschfield	Very thin glacial drift derived from local olivine norite rocks	Yellowish brown medium loam	Inschfield, Insch, Aberdeenshire
Danestone	Boulder till, mainly derived from granite and gneiss, overlying granite	Dark grey light loam	Danestone, Woodside, Aberdeen
Craigiebuckler	Boulder till, derived from local rocks and overlying granitic gneiss	Greyish brown medium loam	Craigiebuckler, Aberdeen

Compared with the other soils Inschfield has a definitely higher silt and clay content and lower sand fractions. In the field, however, only Danestone, one of the granitic soils, appears to be lighter. The ultra basic origin of the Inschfield soil (olivine norite or gabbro) is demonstrated by its low silica and high sesquioxide content. Overhill, although overlying basic rocks, contains a high percentage of silica and its partly basic nature is shown only by its high magnesium and iron contents, compared with the granitic soils. A microscopic examination made by Dr R. Hart showed the presence of minerals from both acidic and basic rock types. Apart from its very low calcium content, the Drumnagair soil resembles that from Overhill both in chemical and mechanical composition.

Table II. *Mechanical analysis—1927 official method*(1)  
*as percentage air-dry soil (< 2 mm.)*

Fraction	Overhill	Drum-nagair	Insch-field	Dane-stone	Craigie-buckler
Coarse sand	33.5	32.4	26.7	41.0	34.4
Fine sand	28.4	31.2	24.2	23.8	25.2
Silt	16.0	15.8	16.7	13.1	14.7
Clay*	15.5	15.0	19.4	10.7	13.6
Air-dry moisture	3.0	2.8	5.8	2.7	3.9
Difference	3.6	2.8	7.2	8.7	8.2
	100.0	100.0	100.0	100.0	100.0
*Including dissolved sesquioxides	1.6	1.1	3.2	1.5	2.0
Loss on ignition	6.8	5.8	13.2	9.7	10.1
Weight of 1 acre of soil (< 2 mm.) to 9 in. in million lb.	2.10	2.05	1.65	1.80	1.75

Table III. *Ultimate analysis as percentage ignited soil*

	Overhill	Drumnagair	Inschfield	Danestone	Craigiebuckler
SiO <sub>2</sub>	71.97	74.27	48.67	74.32	72.03
Fe <sub>2</sub> O <sub>3</sub>	6.26	5.50	10.44	2.28	4.78
Al <sub>2</sub> O <sub>3</sub>	13.30	13.38	27.09	15.35	15.26
TiO <sub>2</sub>	0.82	0.79	3.43	0.73	0.82
CaO	1.63	0.87	3.78	1.48	1.49
MgO	2.26	1.70	3.47	0.86	0.98
Na <sub>2</sub> O	1.49	1.21	1.48	1.90	2.17
K <sub>2</sub> O	1.90	1.78	0.89	2.56	2.58
	99.63	99.50	99.25	99.48	100.11

## METHODS OF INVESTIGATION

The *pH determinations* were carried out on both moist and air-dry samples by means of Biilmann's quinhydrone method, using the procedure recommended by the International Society of Soil Science(3). No drift was observed in any of the soils.

*Exchangeable cation determinations* were made by two methods—the bases were determined by leaching 20 g. of soil to 1 litre with neutral normal ammonium acetate and subsequent determination of calcium, magnesium and, in some cases, sodium and potassium by standard methods, whilst exchangeable hydrogen and exchange capacity were estimated by Parker's method(8). This method was chosen, as it employs a well-buffered neutral leaching solution of high replacing power and therefore gives a value for exchangeable hydrogen comparable with the results obtained for the other exchangeable cations by leaching with ammonium acetate. 10 g. of soil are leached first with 250 c.c. neutral normal barium acetate and the leachate titrated electrometrically with barium hydroxide in order to obtain the hydrogen figure, then leached with normal ammonium chloride, washed with alcohol and the adsorbed ammonia distilled off and determined, in order to get a measure of the exchange capacity.

Results of a series of preliminary experiments using various leaching solutions are recorded in Table IV. These results show the unsuitability of acid leaching solutions, in particular for the Overhill soil. None of the soils contained free calcium carbonate, whilst continued leaching with the neutral solutions gave no increase in the amount of calcium obtained. It must be concluded, therefore, that the acid solutions extract a considerable amount of non-exchangeable calcium.

*Lime requirement determinations* were carried out by the Hutchinson-MacLennan method using calcium bicarbonate(5), by the Kappen hydrolytic acidity method using normal calcium acetate(6) and by a neutral-

lization curve method. The latter was chosen after various modifications had been tried out and was as follows: To a series of 100 c.c. stoppered conical flasks containing 10 g. air-dry soil, different amounts of  $\text{Ca}(\text{OH})_2$  were added. The  $\text{Ca}(\text{OH})_2$  was dissolved in 2 per cent sucrose, as suggested by Smith & Coull(11), and  $N/30$ ,  $N/15$  or  $N/10$   $\text{Ca}(\text{OH})_2$  used as

Table IV. *Preliminary determinations of exchangeable cations on initial composite samples (in m.e. per 100 g. air-dry soil)*

Leaching solution	Calcium				Magnesium		Hydrogen
	$N$ $\text{NH}_4\text{Cl}$	$N$ $\text{CH}_3\text{COONH}_4$	$0.5 N$ $\text{CH}_3\text{COOH}$	$0.05 N$ $\text{HCl}$	$N$ $\text{NH}_4\text{Cl}$	$N$ $\text{CH}_3\text{COONH}_4$	$N$ $(\text{CH}_3\text{COO})_2\text{Ba}$
			17.1 18.9*	10.8*			
Overhill	7.88	7.61	25.9 26.7 13.8 11.3	9.8	3.09	2.61	3.4
Drumnagair	6.50	6.46	7.5	7.6	1.15	0.90	3.3
Inschfield	7.27	7.12	10.8	13.8	1.18	1.08	12.3
Danestone	7.82	7.65	8.9	9.0	0.80	0.59	6.8
Craigiebuckler	9.71	9.58	10.8	11.3	0.90	0.85	5.7

\* Single determinations—good replicates not obtained.

necessary—the total volume not being allowed to exceed 25 c.c., so that a constant soil : solution ratio of 1 : 2.5 could be employed. The volume was made up to 25 c.c. with  $\text{CO}_2$ -free 2 per cent sucrose, and a drop of chloroform added to prevent bacterial decomposition of the sucrose. The flasks were then shaken end-over-end for 24 hours and allowed to stand with occasional shaking for a further 24 hours before the  $p\text{H}$  determinations were made. This period of interaction was found to be the most satisfactory, a fairly definite equilibrium stage being reached, with little or no change occurring during the next 48 hours. 2.5 or 5.0 c.c. increments of  $N/30$   $\text{Ca}(\text{OH})_2$  were employed, depending on the buffering of the soils. Comparison of the  $p\text{H}$  values of air-dried material and the initial points of the neutralization curves showed that the long shaking and the addition of sucrose and chloroform causes little or no change. The curves for the initial samples are shown in Fig. 1. They indicate that Inschfield, the basic soil, is the most highly buffered and that Overhill and Drumnagair are least buffered; the granitic soils, Craigiebuckler and Danestone, occupy an intermediate position. The predominating effect of the organic matter in determining the buffer capacity is suggested by the fact that whilst the loss-on-ignition figures place the soils in this order, the clay-content figures do not.

Further determinations of a physical nature, as well as determinations on subsoil samples of the same soils, will be reported in a subsequent paper by Whelan, who also extended the period of investigation of the



surface samples, and showed that the only change occurring after the first year was a gradual falling off in the effects of the lime.

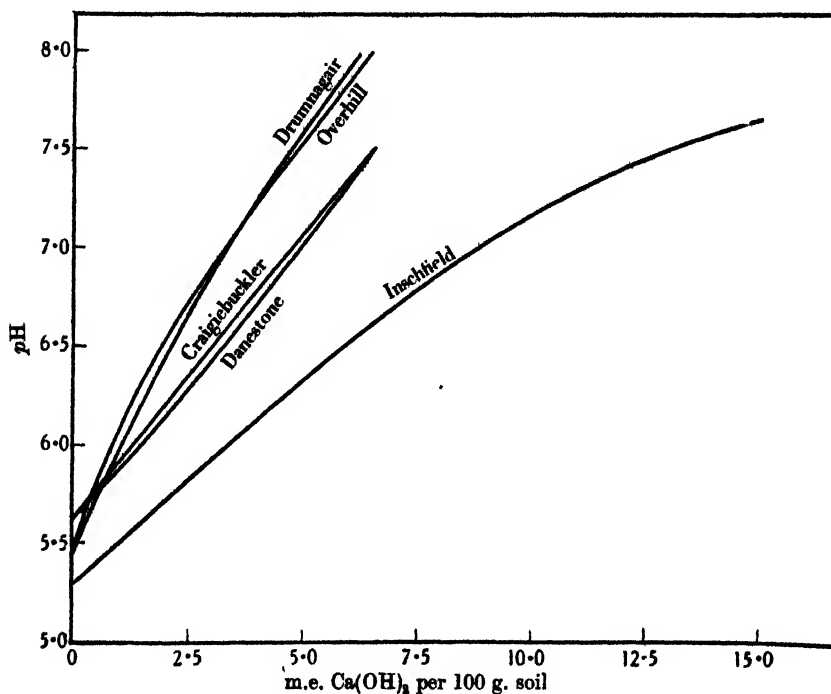


Fig. 1. Neutralization curves: initial samples.

### EXPERIMENTAL RESULTS

Throughout the results which will now be presented, the most important point, both from a scientific and practical aspect, is the markedly different behaviour of the soil derived from the basic igneous parent material, namely Inchfield, compared with the two granitic soils, Danestone and Craigiebuckler, and also with the other two soils, the old red sandstone from Drumnagair and that of mixed basic and acidic origin from Overhill. We shall see that, as might have been expected, the granitic soils show similar responses to the lime applied, and also that Drumnagair and Overhill give almost identical results. Although of different geological origins, they have, as already pointed out, similar mechanical and chemical compositions, and these similarities are again apparent in their response to lime.

(a) *pH determinations*

The *pH* values of the treated and untreated soils will be found in Table V. All the soils fall into the categories of acid or fairly acid. Marked variations at the different times of sampling are not apparent, and apart from the considerable rise in the unlimed Inschfield plots at the third sampling the differences in the unlimed plots are small. This rise is not shown in the *pH* value of the air-dry soil in calcium chloride suspension but only in the moist and air-dry *pH* values determined in aqueous suspension. There is no definite direction of change on air drying, the *pH* values of the initial samples tending to decrease and those of the samples taken a year after liming to rise.

Table V. *pH determinations*

Sample	In aqueous suspension						In calcium chloride suspension		
	Moist soils			Air-dry soils			Air-dry soils		
	Before liming	After 6 months	After 12 months	Before liming	After 6 months	After 12 months	Before liming	After 6 months	After 12 months
Overhill									
Unlimed	6.19	6.15	6.27		5.91	6.43		4.59	4.59
1 ton	6.15	6.66	6.70	5.70	6.51	6.79	4.46	5.15	5.11
2 tons	6.14	7.06	7.03		7.00	7.24		5.56	5.68
Drumnagair									
Unlimed	5.88	5.76	5.97		5.83	6.27		4.61	4.68
1 ton	5.89	6.14	6.48	5.74	6.40	6.63	4.49	5.19	5.02
2 tons	5.87	7.05	6.96		7.12	7.07		5.89	5.42
Inschfield									
Unlimed	5.37	5.54	5.78		5.37	5.89		4.51	4.56
1 ton	5.41	5.83	6.15	5.30	5.88	6.15	4.38	4.85	4.80
2 tons	5.40	6.24	6.75		6.40	6.70		5.27	5.51
Danestone									
Unlimed	5.69	5.82	5.86		5.87	5.92		4.59	4.59
1 ton	5.74	6.07	6.20	5.62	6.11	6.15	4.47	4.80	4.82
2 tons	5.74	6.39	6.55		6.44	6.61		5.17	5.25
Craigiebuckler									
Unlimed	5.90	5.79	5.76		5.79	5.84		4.51	4.50
1 ton	5.89	6.04	6.12	5.64	6.09	6.08	4.52	4.84	4.73
2 tons	5.93	6.39	6.46		6.34	6.46		5.13	5.10

Table VI gives the differences between the *pH* values in aqueous suspension of the moist soils from limed and unlimed plots. The full effect of the lime on the *pH* value has been obtained after only 6 months at Overhill and Drumnagair, the least buffered soils, whilst at Danestone and Craigiebuckler the *pH* differences between limed and unlimed plots increase during the second 6 months. At Inschfield, the most highly buffered soil, there is a marked increase in neutralization in the 2-ton plots during the second half year. The results for the air-dry *pH* values

generally show corresponding changes. The irregularity in the Drumnagair 1-ton plots occurs only in the moist samples.

Table VI. *pH changes caused by the application of lime (pH values determined in aqueous suspension of moist soils)*

	1 ton CaO		2 tons CaO	
	After 6 months	After 12 months	After 6 months	After 12 months
Overhill	0.51	0.43	0.91	0.76
Drumnagair	0.38	0.51	1.29	0.99
Inschfield	0.29	0.37	0.70	0.97
Danestone	0.25	0.34	0.57	0.69
Craigiebuckler	0.25	0.36	0.56	0.66

The greatest absolute changes, as well as the quickest response, have occurred at Overhill and Drumnagair, with increases in pH value of 0.5 pH units per ton of lime applied. The granitic soils, Danestone and Craigiebuckler, show only half this response. The buffering of the soils appears to be such that the change in pH value brought about by 2 tons of lime is approximately twice as great as that brought about by 1 ton, in other words, there is a logarithmic relationship between the amount of lime applied and the change in hydrogen-ion concentration.

(b) *Exchangeable cation determinations*

The results for the samples taken 6 and 12 months after liming are given in Tables VII and VIII respectively. In none of the limed soils were there appreciable quantities of calcium carbonate. The best representation of the changes occurring in the base status is given by the figures for the relative proportions of the exchangeable cations, together with that for base exchange capacity. The latter varies from 11 m.e. per 100 g. for the Drumnagair soil to 22 m.e. for Inschfield, whilst the amounts of basic cations in the unlimed samples are approximately constant. Thus Inschfield contains almost four times as much exchangeable hydrogen as does Drumnagair—a result which would not be expected from the pH determinations. A tendency of the Overhill soil to dry into hard lumps may be due to its relatively high exchangeable magnesium content.

The relative proportions of the basic cations are similar to those of other investigators quoted by Smith (10), but these figures have not been included as, when hydrogen, the most important cation from the soil acidity point of view, is excluded from the calculation, the real effects of the lime on the base status are masked by the initial preponderance of

Table VII. *Exchangeable cations in samples taken 6 months after liming (as m.e. per 100 g. air-dry soil and as percentages)*

	Soil	Exchangeable cations					Total basic ions			Ex- change capacity Parker's method	Percentage of total cations					Per- centage saturation Parker's method
		Ca	Mg	Na	K	H	Ca + Mg + Na + K				Ca	Mg	Na	K	H	
							Parker's method									
Overhill	Unlimed	7.8	2.9	0.20	0.15	3.5	11.1	10.1	13.6	53.6	20.0	1.4	1.0	24.0	74.1	
	1 ton	9.0	3.0	0.24	0.14	2.9	12.4	10.6	13.5	59.1	19.4	1.6	0.9	19.0	78.4	
Drumsgair	Unlimed	10.9	3.2	0.24	0.14	1.8	14.5	11.8	13.6	67.3	19.2	1.5	0.9	11.1	86.8	
	1 ton	6.4	0.9	0.21	0.26	3.4	7.8	7.7	11.1	57.4	8.1	1.9	2.3	30.2	69.5	
Ineschfield	Unlimed	8.4	1.0	0.24	0.25	2.2	9.9	9.0	11.2	69.5	8.5	2.0	2.1	17.9	80.7	
	2 tons	10.4	1.1	0.24	0.25	0.9	12.0	10.3	11.2	80.6	8.4	1.9	1.9	7.2	91.7	
Daneestone	Unlimed	7.2	1.2	0.48	0.13	12.8	8.9	8.8	21.6	33.1	5.3	2.2	0.6	58.8	40.8	
	1 ton	9.9	1.4	0.55	0.11	10.1	12.0	11.9	22.0	45.0	6.1	2.5	0.5	45.8	54.1	
Craigiebuckler	Unlimed	13.0	1.5	0.57	0.13	7.8	15.2	14.4	22.1	55.5	6.6	2.5	0.6	33.8	64.9	
	1 ton	7.6	0.7	0.24	0.17	6.9	8.7	7.7	14.7	48.8	4.2	1.5	1.1	44.4	52.7	
Craigiebuckler	Unlimed	8.8	0.8	0.24	0.18	5.6	10.0	9.3	14.8	56.7	4.9	1.5	1.2	35.7	62.5	
	2 tons	10.8	0.9	0.24	0.16	3.6	12.2	11.3	14.9	68.4	5.9	1.5	1.0	23.2	75.5	
Craigiebuckler	Unlimed	9.8	1.0	0.32	0.12	6.1	11.2	10.4	16.5	56.6	5.6	1.8	0.7	35.3	63.0	
	1 ton	11.6	1.2	0.32	0.11	4.9	13.2	11.9	16.8	63.8	6.5	1.8	0.6	27.3	70.8	
Craigiebuckler	Unlimed	13.4	1.3	0.34	0.10	3.4	15.1	13.4	16.9	72.3	6.9	1.8	0.5	18.5	79.7	

Table VIII. Exchangeable cations in samples taken 12 months after liming (as m.e. per 100 g. air-dry soil and as percentages)

Soil	Exchangeable cations			Total basic ions Ca + Mg + Na + K	Parker's method	Exchange capacity Parker's method	Percentage of total cations			Parker's method	Percentage saturation
	Ca	Mg	H				Ca	Mg	H		
Overhill Unlimed 1 ton	6.8	2.4	4.5	9.5	10.6	15.1	47.7	17.1	31.8	70.4	70.4
	8.6	2.7	3.6	11.6	11.4	14.9	56.4	17.7	23.4	76.2	76.2
Drummagair Unlimed 1 ton	11.3	3.0	1.9	14.6	13.4	15.2	68.3	18.2	11.3	87.8	87.8
	5.1	0.9	4.1	6.4	8.3	12.4	48.4	8.2	38.9	67.1	67.1
Inschfield Unlimed 2 tons	6.6	1.0	3.2	8.0	9.3	12.4	58.9	8.5	28.4	76.1	76.1
	8.4	1.1	1.5	10.0	11.0	12.5	73.5	9.8	12.7	88.3	88.3
Danestone Unlimed 1 ton	7.4	1.0	12.4	9.0	9.1	21.5	34.5	4.6	57.9	42.3	42.3
	9.6	1.2	9.3	11.4	12.3	21.7	46.2	5.6	45.1	56.8	56.8
Craigiebuckler Unlimed 2 tons	14.7	1.5	6.2	16.9	15.9	22.1	63.7	6.6	26.9	71.8	71.8
	7.1	0.6	7.5	8.1	6.1	13.6	45.6	3.9	47.8	45.1	45.1
Craigiebuckler Unlimed 1 ton	7.6	0.7	6.1	8.8	7.7	13.8	51.3	5.0	41.0	55.8	55.8
	10.3	1.0	3.9	11.6	9.9	13.8	70.7	6.6	26.9	71.7	71.7
Craigiebuckler Unlimed 2 tons	8.7	0.8	7.0	10.0	9.2	16.2	51.4	4.8	41.3	56.7	56.7
	9.5	0.9	5.8	10.8	10.5	16.4	57.2	5.3	34.9	64.4	64.4
Craigiebuckler Unlimed 2 tons	12.0	1.2	3.5	13.6	12.9	16.3	70.3	6.9	20.3	78.8	78.8

calcium. In fact, it may be stated generally that for unsaturated soils of podzolic and brown earth types, results for the relative proportions of the basic cations are of little value unless accompanied by the exchange capacity and degree of saturation figures. The base status is best defined by the exchange capacity together with the relative proportions of all the exchangeable cations including hydrogen.

In all the soils except Inschfield there has been an appreciable fall in the calcium content of the untreated plots over the winter, whilst exchangeable hydrogen shows corresponding increases. Calculations based on the decreases in exchangeable calcium would appear to indicate a loss of 250 and 500 lb. CaO per acre from the top 9 in. of the granitic soils, the greater loss being from the soil which was fallow over the winter. Lysimeter results for a granitic soil near Aberdeen(2) show a removal of 100 lb. CaO through a 40 in. column of soil.

*Effects of lime on the base status.* The increases in exchangeable calcium and magnesium content are generally slightly greater than the corresponding decreases in hydrogen. Part of the difference may be accounted for by the increase of base exchange capacity on liming noted previously by Pierre & Scarseth(9), Walker, Brown & Young(12) and others, whilst part may be due to solution of traces of  $\text{CaCO}_3$  remaining in the soils. Sodium and potassium show no appreciable changes. The greatest absolute increase in exchangeable calcium occurs at Inschfield, where about 70 per cent of the lime applied has become exchangeable. For the other four soils the proportion is about 50 per cent. The greatest displacement of hydrogen and entry of calcium takes place in the soils with the highest initial hydrogen content. This is in accordance with the results of Hissink(4), who stated that the less saturated a soil was, the greater the percentage entry of calcium.

Drumnagair has been brought most nearly to saturation. Inschfield is still the most unsaturated after the addition of 2 tons of lime although it shows the greatest absolute reduction in exchangeable hydrogen. Drumnagair and Overhill are 80–90 per cent saturated at pH 7, a result in accordance with the findings of Walker, Brown & Young(12).

After allowance is made for the changes in the untreated plots during the second 6 months, there is during this period little further change in the base status of the soils other than Inschfield attributable to the action of the lime. The relative proportion of calcium has in each case decreased and that of hydrogen increased, when the October and March results are compared. In the Inschfield soil, where there is no appreciable change in the untreated samples, the results point to a continuation of

the neutralization process during the second 6 months in the 2-ton plots.

In the granitic soils the calcium : magnesium ratio of approximately 11 is unchanged on liming; for the other soils, with Ca : Mg ratios smaller than that of the lime applied, the ratio increases on liming. The percentage entry of magnesium is in general lower than that of calcium, in agreement with the order of ease of entry by displacement (13).

*(c) Lime requirement determinations*

The two single value "lime-requirement" methods will be considered first. The results, in terms of m.e. of hydrogen which must be neutralized, will be found in Table IX. The two methods give results in fairly good agreement, although the Kappen method is less satisfactory in accounting for the lime applied. The Hutchinson-MacLennan method assumes the lime requirement to be equivalent to the amount of hydrogen found, whilst Kappen advises a factor of 2 in the conversion to tons of lime required per acre.

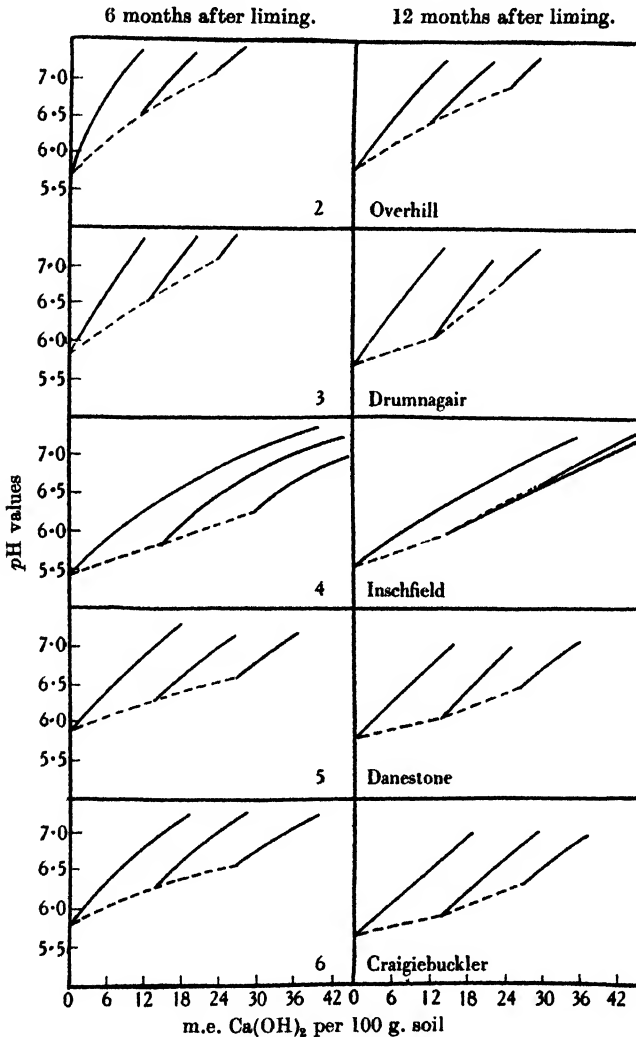
For a lime requirement method to be efficient it ought to show a decrease in lime requirement equivalent to the amount of lime added to the treated plots. The actual decreases found are given in Table X.

Thus for the Hutchinson-MacLennan method a factor of 3 is necessary for the Inchfield soil and one of 5 for the other four soils in order to account for the lime applied. The Kappen factor of 2, included in the results of Table X, is obviously inadequate.

Much more information regarding the behaviour of the soils on the application of lime is obtained from Figs. 2-6, which show the results obtained in the neutralization curve determinations. The flatter curves represent the field responses and the three steeper curves in each diagram the effect of adding lime in the laboratory to the samples which had received no lime, 1 ton and 2 tons respectively. The liming factor at any pH value is the ratio of the horizontal intercepts cut off by the field response curve and the laboratory curve. The lime requirement is then obtained by multiplying the amount of  $\text{Ca}(\text{OH})_2$  needed to obtain that pH value in the laboratory by this factor and converting the result into tons CaO per acre, using the volume-weight correction, which has been applied in the curves.

Overhill and Drumnagair show similar responses, except that the effect of the lime seems to decrease to a greater extent in the second 6 months in the latter. Danestone and Craigiebuckler, the granitic soils, again show similar responses, both in field and laboratory. The Inchfield

soil does not resemble the others in its behaviour, especially during the second 6 months. In the samples taken 6 months after liming the curve



Figs. 2-6. Neutralization curves after liming.

for the 2-ton plots is practically a continuation of the field response curve, indicating a low liming factor. In the March samples a further reduction in acidity has occurred in the limed plots. The 1-ton and 2-ton curves coincide: thus the additional ton of lime added in the field has had an



Table IX. *Lime requirement (m.e.  $H^+$  per 100 g. air-dry soil)*

Soil	Kappen method			Hutchinson-MacLennan method		
	Before liming	After 6 months	After 12 months	Before liming	After 6 months	After 12 months
Overhill						
Unlimed		1.95	2.42		1.96	2.30
1 ton	2.10	1.39	1.77	2.06	1.02	1.39
2 tons		1.00	1.19		0.39	0.51
Drumnagair						
Unlimed		1.88	2.25		1.78	1.47
1 ton	1.96	1.28	1.94	1.69	0.98	1.13
2 tons		0.72	1.45		0.00	0.41
Inschfield						
Unlimed		4.12	4.62		5.36	5.57
1 ton	4.37	3.20	3.58	5.57	4.02	4.15
2 tons		2.46	2.33		2.67	2.15
Danestone						
Unlimed		2.70	3.46		2.54	3.33
1 ton	3.11	2.35	2.98	2.83	1.92	2.62
2 tons		1.85	2.19		1.37	1.73
Craigiebuckler						
Unlimed		3.18	3.88		2.92	3.50
1 ton	3.05	2.54	3.16	2.75	2.13	2.64
2 tons		2.09	2.35		1.72	1.75

Table X. *Decrease in lime requirement caused by the addition of lime (as tons  $CaO$  per acre)*

Method	Hutchinson-MacLennan				Kappen			
	1 ton		2 tons		1 ton		2 tons	
Amount applied	After 6 months	After 12 months	After 6 months	After 12 months	After 6 months	After 12 months	After 6 months	After 12 months
Sample								
Overhill	0.22	0.22	0.39	0.44	0.28	0.31	0.48	0.60
Drumnagair	0.19	0.09	0.44	0.27	0.31	0.16	0.59	0.40
Inschfield	0.28	0.35	0.61	0.85	0.46	0.52	0.83	1.15
Danestone	0.16	0.18	0.36	0.40	0.18	0.25	0.43	0.64
Craigiebuckler	0.14	0.21	0.24	0.43	0.32	0.37	0.54	0.77

effect equal to that brought about by the equivalent amount of lime as  $Ca(OH)_2$  in the laboratory. It follows that the liming factor is unity at this point.

The liming factors obtained are collected in Table XI. Except for Inschfield, they indicate a factor of about 3 for neutralization to pH 6.0 and of about 2.5 when a pH value of 6.5 is desired. At Inschfield, on the other hand, the factor is 2.0 for a pH value of 6.0 and only 1.5 for a pH value of 6.5, whilst the liming factor for a further application of lime after the first ton dressing is unity—a second ton has a response equal to the theoretical laboratory response. The slower action of the lime at Inschfield is well demonstrated.

Table XI. *Liming factors*

Aqueous pH value to be obtained	After 6 months			After 12 months		
	6.0	6.5	7.0	6.0	6.5	7.0
Overhill						
Unlimed	2.8	2.8	2.9	2.7	2.4	2.3
1 ton	—	—	2.2	—	2.0	2.0
Drumnagair						
Unlimed	3.0	2.8	2.6	(5.4)	3.0	—
1 ton	—	—	2.1	—	1.7	—
Inschfield						
Unlimed	2.9	2.2	—	1.9	1.5	—
1 ton	2.2	2.1	—	—	1.0	—
Danestone						
Unlimed	3.3	3.3	—	3.6	3.1	—
1 ton	—	3.1	—	—	2.6	—
Craigiebuckler						
Unlimed	3.1	2.8	—	3.2	2.5	—
1 ton	—	2.8	—	3.0	2.4	—

It is interesting to note that the ratios of the liming factors for the first four soils and for Inschfield, viz. 2.5 : 1.5, is the same as the ratio 5 : 3 found for the Hutchinson-MacLennan method to account for the lime applied. Whilst the ratios of the factors are the same, however, the Hutchinson-MacLennan method only accounts for half as much of the lime as does the neutralization curve method, so that from this point of view the latter is a definite advance. The Kappen method is least satisfactory of all in this respect.

The neutralization curve method gives information with regard to any pH value which it may be desired to reach, provided the liming factor for the soil type is known—an advantage which the single value methods do not possess.

The soils at Overhill, Drumnagair, Danestone and Craigiebuckler are all podzolized to some extent, whilst profile investigations (carried out by the author and reported by W. G. Ogg<sup>(7)</sup>) on soils derived from basic igneous parent materials near Inschfield show no evidence of podzolization. Such soils show a definite zone of exchangeable calcium accumulation at about 50 cm. It is possible that the explanation of the low liming factor and the other differences shown by the Inschfield soil may be found in these genetical differences.

#### SUMMARY

The effects of lime on five soils representative of types from north-east Scotland have been studied and different lime requirement methods compared.

Laboratory analyses including mechanical and ultimate chemical analyses, pH, exchangeable cation and lime-requirement determinations were carried out on samples taken before and at two intervals of 6 months after the application of lime.

In some cases the maximum effect of the lime applied occurs within 6 months, in others neutralization is continued during the second 6 months.

The change in pH value varies, according to the soil type, from 0.5 to 1.0 pH units per ton of calcium oxide applied per acre.

The liming factor, as shown by the neutralization curve lime requirement method, also depends on the soil type. In four of the soils examined, it lies between 2.5 and 3.0: in the fifth, a geologically basic soil, it is only 1.5. Similar differences are shown in the lime requirement determinations by the Hutchinson-MacLennan and Kappen methods. These methods are much less satisfactory in accounting for the lime applied.

Exchangeable cation determinations show that, in the first four soils noted above, 50 per cent of the calcium applied has entered the exchange complex, in the other, 70 per cent.

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